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The Neuroendocrinology of Stress: Its Relation to the Hormonal Milieu, Growth, and Development

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Life exists by maintaining a complex dynamic equilibrium, termed "homeostasis," that is constantly challenged by intrinsic and extrinsic adverse forces, the stressors.[1] "Stress," a term borrowed from physics first by W. Cannon and subsequently used by H. Selye, refers to factors threatening homeostasis. The human mind and body react to stress by activating a complex repertoire of adaptive central nervous system (CNS) and peripheral responses, the familiar "fight-or-flight" response.[2] Successful adaptive responses are generally specific to a stressor, but can become relatively nonspecific when a stressor of any kind exceeds a threshold magnitude. Alterations in the ability of the organism to respond to stressors, with the responses being either excessive or inadequate in magnitude and duration, may lead to disease.

The adaptive response of an individual to stress is determined by a multiplicity of genetic and environmental factors, with development being an important consideration.[3] Indeed, prenatal life, infancy, childhood, and adolescence are critical periods characterized by increased vulnerability to stressors.[4] If stresses are excessive and/or these adaptive responses are prolonged, personality development and, hence, behavior may be disturbed. Adverse consequences on physiologic functions, including growth, metabolism, reproductive function, and the inflammatory/immune response, may result. Thus, stressors in early life may lead to developmental, psychiatric, growth, metabolic, reproductive, and immunologic disorders.

In this brief article, the reader will be introduced to the neuroendocrinology of the stress response; the stress system itself; the regulation of affect; and the effects of stress on endocrine functions, with emphasis on the hormonal milieu, growth, and the effect of stress on the immune system. The interdigitation of these are emphasized.

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

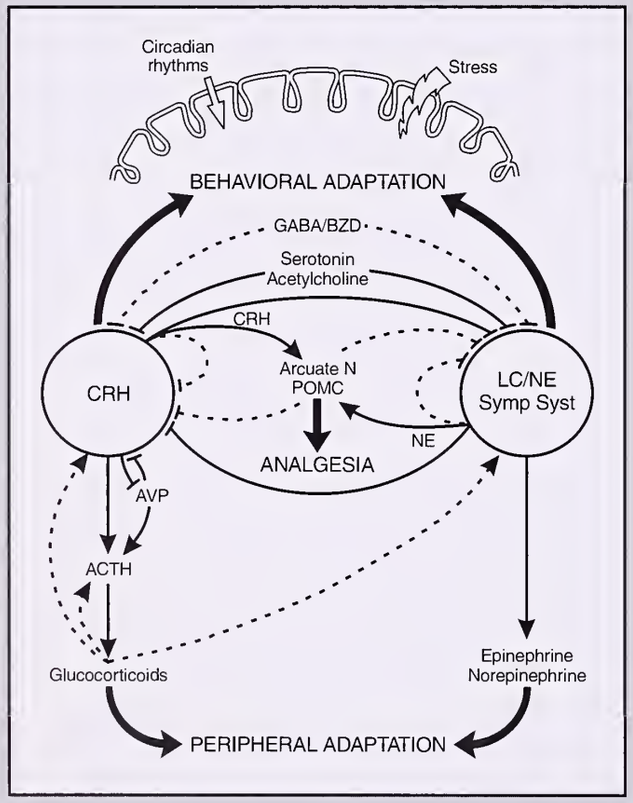
In This Issue

NEUROENDOCRINOLOGY OF THE STRESS RESPONSE

The adaptive response to stress is characterized by both behavioral and physical changes.[2,4] These include increased arousal and alertness, heightened attention, and suppression of "vegetative" functions, which include sexual activity, feeding behavior, and growth. There is redirection of energy, ie, oxygen and nutrients, to the body site that is stressed and to the CNS, where it is most needed. The adaptive response is coordinated by the central and peripheral components of the stress system. The central components constantly receive information from higher and lower centers of the CNS, from the periphery of the organism, and from the environment. These then are integrated to help coordinate the dynamic equilibrium of the organism.

As diagramed in Figure 1, the central coordinators of the stress system include the parvicellular ("composed of small cells") corticotropin-releasing hormone (CRH) neurons; the arginine-vasopressin (AVP) neurons of the paraventricular nuclei (PVN) in the hypothalamus; the CRH neurons of the paragigantocellular and other nuclei in the medulla; and the catecholaminergic neurons of the locus ceruleus (LC) and other cell groups in the medulla and the pons. The hypothalamic-pituitary-adrenal (HPA) axis and the efferent sympathetic/adrenomedullary system represent the peripheral limbs of the central coordinating body. Reciprocal neural connections exist between the CRH and catecholaminergic neurons of the CNS, and there are autoregulatory ultra-short negative feedback loops exerted by CRH on the CRH neurons and on the catecholaminergic neurons exerted by norepinephrine via collateral fibers and presynaptic receptors. Both CRH and noradrenergic neurons are stimulated by serotonin and acetylcholine, and inhibited by glucocorticoids, α-aminobutyric acid (GABA), α-melanocyte-stimulating hormone (MSH), and opioid peptides.

Figure 1



A simplified representation of the central and peripheral components of the stress system, their functional interrelations, and their relations to other CNS systems involved in the stress response. LC = locus ceruleus, NE = norepinephrine. Activation is represented by solid lines, and direct or indirect inhibition by dashed lines. Adapted with permission from Chrousos and Gold.[2]

Parvicellular neurons of the PVN produce CRH and AVP (Figure 1), and reciprocally innervate and are innervated by opioid peptide (pro-opiomelanocortin [POMC])-producing neurons of the arcuate nucleus of the hypothalamus. Thus, activation of the stress system stimulates hypothalamic POMC-peptide secretion, which reciprocally inhibits the activity of the stress system and, in addition, produces analgesia through projections to the hindbrain and spinal cord. CRH, of course, stimulates and also is permissive for pituitary ACTH secretion. On the other hand, AVP is a potent synergistic factor of CRH, but it has very little ACTH secretagogue activity by itself. During stress, AVP and CRH secretion into the hypophyseoportal system results in increased ACTH and, hence, cortisol release into the systemic circulation. Additional CRH, AVP, ACTH, and cortisol secretagogues are recruited during the various types of stress, further potentiating the activity of the HPA axis. These include the inflammatory cytokines, angiotensin II, and other mediators.

Glucocorticoids are the final effectors of the HPA axis and participate in the control of homeostasis in a multifaceted way. They play a key regulatory role in the basal activity of the HPA axis and in the termination of the stress response by exerting negative feedback at the CNS components of the stress system. A major function of glucocorticoids is the protection of the organism from the consequences of excessive adaptive responses. An example of such a role is the profound anti-inflammatory/immunosuppressive activity of glucocorticoids.

The sympathetic division of the autonomic nervous system provides a rapidly responding mechanism that controls mostly the acute response of the organism to a stressor. Peripherally, it widely innervates vascular smooth muscle cells, as well as

the kidneys, gut, and many other organs, and the adrenal medulla. In addition to acetylcholine, norepinephrine, and epinephrine, the sympathetic and the parasympathetic divisions of the autonomic nervous system secrete a variety of neuropeptides, such as CRH, neuropeptide Y (NPY), somatostatin (STS), galanin, enkephalin and neurotensin, adenosine triphosphate (ATP), prostanoids, and nitric oxide (NO).

STRESS SYSTEM AND THE REGULATION OF AFFECT

Activation of the stress system occurs in diametrically opposed situations, such as pleasure and dysphoria.[2,4] Indeed, novelty seeking and self-driven activation of the stress system — an important component of human development — is associated with pleasure if the response is adaptive and the individual has a sense of control. In contrast, stress may lead to dysphoria if the response is maladaptive and if the individual has the perception of no control. The teleology of these phenomena is obvious, for this is how an individual respectively seeks favorable changes and avoids, or learns to avoid, situations that may be detrimental to existence. The crucial nature of the stress system in human survival is underscored by the fact that it is activated not only by novelty but also by both feeding and sexual activity, sine qua non functions for self-preservation and species preservation.

The mechanisms regulating the stress-activated mood response are complex and poorly understood. It appears that the stress system has reciprocal interactions with at least 3 other elements of the CNS that participate in the regulation of emotions: (1) the mesocortical and mesolimbic dopamine systems, which include the prefrontal cortex and nucleus accumbens and which are involved in anticipatory and motivational/reinforcement and reward phenomena, respectively; (2) the amygdala/hippocampus complex, which is involved in emotional stressors such as conditioned fear; and (3) the arcuate nucleus opioid peptide-secreting neurons, which alter sensitivity to pain and perhaps influence the emotional tone of an individual.

Several emotional disorders may represent dysregulation of the generalized stress response. Thus, excessive and prolonged activity of the stress system characterizes melancholic depression, whose cardinal symptoms are hyperarousal (anxiety), suppression of feeding (anorexia) and sexual behaviors (loss of libido), and excessive and prolonged redirection of energy (tachycardia, hypertension, carbohydrate intolerance, dyslipidemia). All of these are extremes of the classic manifestations of the "generalized" stress response.[5] The dysphoria that accompanies this condition may represent a response to a perceived uncontrollable stressor and could be due to tachyphylaxis of the mesocorticolimbic system in response to chronic activation by the stress system, while the obsessiveness that characterizes depressive individuals appears to represent a maladaptive increase in the attention span.[5]

Both the HPA axis and the sympathetic system are chronically activated in melancholic depression, in which hyperarousal of the stress system occurs, producing increased CRH. Increased CRH causes or is associated with insomnia, depressed mood, inability to concentrate, decreased appetite, decreased libido, and weight loss. Melancholic depression rarely afflicts children and adolescents. However, chronic activation of the HPA axis and/or the sympathetic system has been shown in a host of other conditions that afflict children, adolescents, and young adults in a major fashion, including malnutrition, anorexia nervosa, panic disorder, obsessive-compulsive neurosis, chronic active alcoholism, alcohol and narcotic abuse, excessive exercising, and post sexual abuse traumatic disorder.[2,6] Animal studies are confirmatory of the association between increased CRH secretion, chronic activation of the HPA axis, and affective disorders. For example, traumatic separation of infant rhesus monkeys and laboratory rats from their mothers causes behavioral agitation and increased CRH, ACTH, and cortisol responses to stressors throughout their lives.[7,8] This is in accordance with human studies showing that melancholic depression has a strong association with precipitating environmental stressors.[9]

Interestingly, the HPA axis and/or the sympathetic system appear hypoactive in several pathologic states, including seasonal affective disorder, in the postpartum period, and the period following the cessation of smoking, as well as in the chronic fatigue and fibromyalgia syndromes — all dysphoric, hypoarousal states.[2,10,11] These conditions are usually characterized by an increase in appetite, weight gain, somnolence, and fatigue — manifestations compatible with low CRH secretion and hypoactivation of the mesocorticolimbic system.

There is general agreement that adolescence is a challenging period of life during which significant physical, psychological, and social changes take place.[12] Adolescents are in a chronic state of "threatened homeostasis," and their adaptive responses are crucial for a successful and happy adulthood. Dysregulation of the stress system in adolescence by the mechanisms indicated above could be the reason behind the emergence of a number of disorders during this period, including

depression, eating disorders, and substance abuse. Recently, it became apparent that it is the "atypical" form of depression that primarily afflicts adolescents and that this form appears to be genetically distinct from the melancholic form usually seen in adults.[13,14]

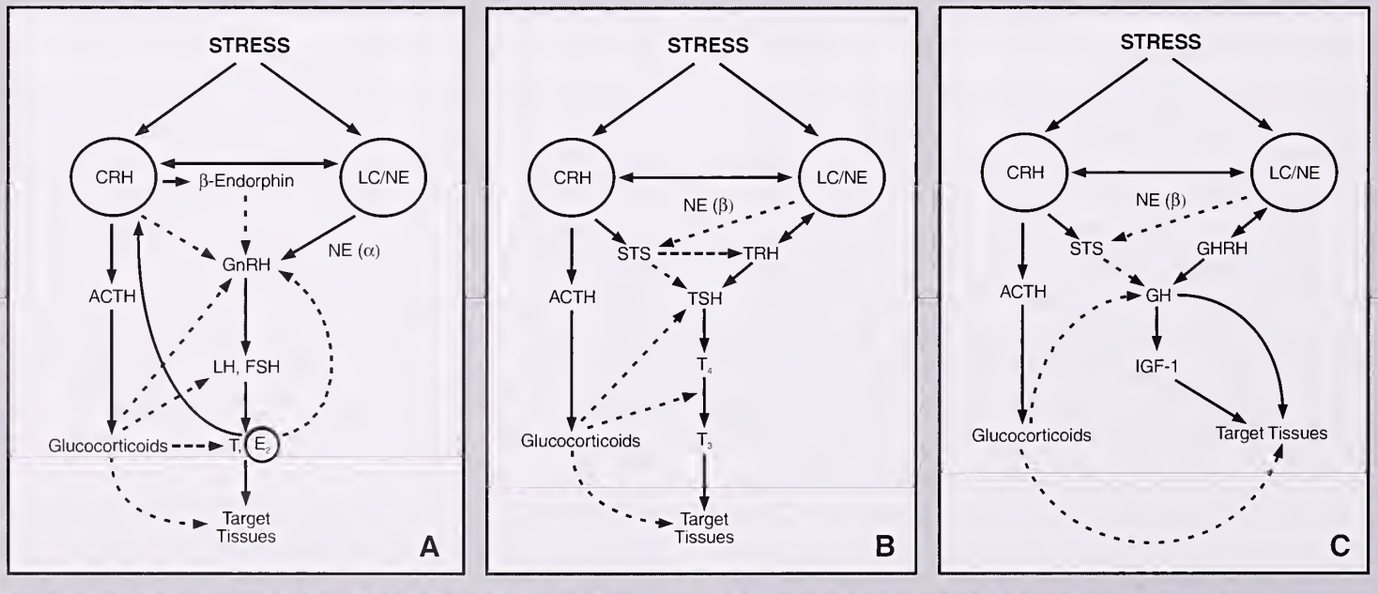
STRESS AND ENDOCRINE FUNCTIONS: EFFECTS ON THE HORMONAL MILIEU

Reproduction and growth are profoundly influenced by stress.[2,4] The reproductive axis is inhibited at all levels by various components of the stress system (Figure 2A). Thus, CRH suppresses the secretion of gonadotropin hormone-releasing hormone (GnRH) by arcuate neurons of the hypothalamus, either directly or via the stimulation of arcuate POMC peptide-secreting neurons. Moreover, glucocorticoids exert inhibitory effects at the level of the GnRH neuron, the pituitary gonadotrope, and the gonads themselves, and render target tissues of sex steroids resistant to these hormones. Suppression of gonadal function caused by chronic HPA axis activation has been demonstrated in highly trained runners of both sexes and ballet dancers. These subjects have increased evening plasma cortisol and ACTH, increased 24-hour urinary free cortisol excretion, and blunted ACTH responses to exogenous CRH. Males have low levels of luteinizing hormone (LH) and testosterone, and females have amenorrhea. Characteristically, obligate athletes go through withdrawal symptoms and signs if for any reason they have to discontinue their exercise routine. This syndrome is possibly the result of withdrawal from the daily exercise-induced activation of the dopaminergic system and/or elevation of opioid peptides.

The interaction between CRH and the gonadal axis appears to be bidirectional.[15] We recently demonstrated the presence of estrogen-responsive elements in the promoter area of the CRH gene and direct stimulatory estrogen effects on CRH gene expression. This finding implicates CRH and, therefore, the HPA axis as a potentially important target of ovarian steroids and a potential mediator of gender-related differences in the stress response. Indeed, Kirschbaum et al[16] recently showed that estrogen administration to normal male volunteers resulted in excessive stress system responses to mental stress.

In parallel to the gonadal axis, the stress system suppresses function of the thyroid axis (Figure 2B).[2,4] During stress, there is suppressed secretion of thyrotropin and decreased conversion of the relatively inactive thyroxine (T_4) to the potent triiodothyronine (T_3) in peripheral tissues. This situation is similar to what is observed in the euthyroid sick syndrome, a phenomenon that serves to conserve energy during stress. The mediators of these changes in thyroid function include glucocorticoids,

Figure 2



A schematic representation of the interactions between the stress system and other neuroendocrine axes: (A) the reproductive axis; (B) the thyroid axis; and (C) the growth axis. Note that the LC/NE system provides positive stimulation to all 3 axes; this effect is overcome by the inhibitory effects of the HPA axis during stress. Hypoactivity of the LC/NE in several human states, such as atypical depression and the chronic fatigue/fibromyalgia syndromes, may be responsible for the mild central hypogonadism, hypothyroidism and hyposomatotropism observed in such states. Adapted with permission from Chrousos and Gold.[2]

which suppress thyrotropin secretion; the activity of the peripheral enzyme 5'-deiodinase, which converts L-T_4 to L-T_3; somatostatin, which suppresses both TRH and thyrotropin; and, in the case of inflammatory stress, the cytokines, tumor necrosis factor-α (TNFα), interleukin 1 (IL-1), and interleukin 6 (IL-6), all of which activate CRH secretion and also directly inhibit the 5'-deiodinase. Accordingly, patients with melancholic depression, anorexics, and chronically ill patients have significantly lower thyrotropin and T_3 hormone concentrations than controls.

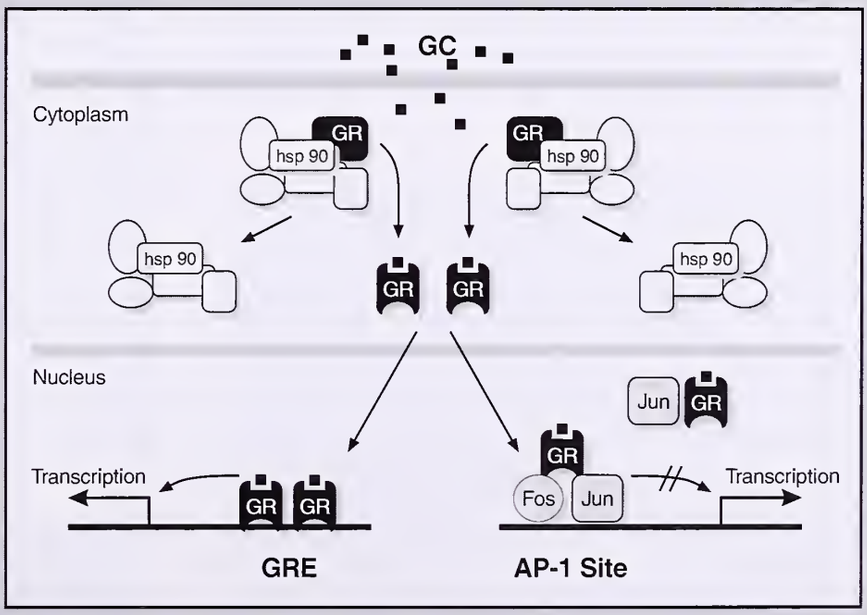
STRESS AND ENDOCRINE FUNCTIONS: EFFECTS ON GROWTH AND DEVELOPMENT

The growth axis also is inhibited at many levels during stress (Figure 2C).[2,4] Thus, prolonged activation of the HPA axis leads to suppression of growth hormone (GH) release and inhibition of insulin-like growth factor 1 (IGF-1) effects on its target tissues. CRH-induced increases of somatostatinergic tone have been implicated as a potential mechanism of stress-induced chronic suppression of GH secretion. It is noteworthy that acute elevations of GH concentrations in plasma occur at the onset of the stress response and after acute administration of glucocorticoids, presumably through stimulation of the GH gene by its glucocorticoid-responsive elements (GREs).

One of the major ways by which the HPA axis inhibits growth is by glucocorticoid-induced resistance of target tissues to IGF-1. Indeed, children with Cushing syndrome have delayed or arrested growth and lose an average of 7.5 to 8.0 cm of their final height as adults.[17-19] The molecular mechanism by which glucocorticoids render tissues resistant to IGF-1 and other growth factors is complex.[20] A major mechanism is the inhibition of growth factor third messengers, such as the cJun-Fos heterodimer or AP-1 transcription factor, by protein-protein interactions between this factor and the ligand-bound, "activated" glucocorticoid receptor (Figure 3).

Psychosocial short stature (PSS) is a term describing severe childhood or adolescent short stature and/or delayed puberty due to emotional deprivation or psychological harassment.[21] This topic is discussed in an abstract and letters to and from the editor on page 8 of this issue. Decreased GH secretion that is reversible after separation of the child from the responsible environment is a very frequent finding in this condition.[22] PSS is also associated with a variety of behavioral abnormalities, such as depression and bizarre eating behaviors. This condition was first studied in infants in foundling homes or orphanages who failed to thrive or had decreased growth. It was hypothesized that failure to thrive and/or grow resulted from lack of attention and stimulation and/or deficient nutrition. Later it was shown that weight gain was independent of food intake, whereas with a caring and attentive environment, growth advanced and the psychological profile improved. In addition to low GH secretion, these patients often had decreased cortisol secretion and/or a dysfunctional thyroid axis — all manifestations compatible with a hyperfunctioning stress system.[23]

Figure 3



A simplified model of glucocorticoid-mediated transcriptional modulation. Hormone binding causes dissociation of the glucocorticoid receptor/hsp complex and nuclear translocation of the ligand-bound receptor. Within the nucleus, the "activated" receptor can act in 2 ways: As indicated on the left, it can bind to glucocorticoid responsive elements (GREs) in the regulatory region of target genes. This interaction causes either stimulation or, less frequently, inhibition of transcription. As indicated on the right, the activated glucocorticoid receptor can also interact with, and inhibit the transactivational activity of, other transcription factors important for growth or immune function, such as, respectively, the cJun-Fos heterodimer and the NF-κB transcription factor. From Bamberger CM, et al. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocrine Reviews* 1996;17(3):247; © The Endocrine Society.

We have reported a nonhuman primate model in which the quality of parental care correlated well with infant and child growth and development.[24] Improvement of care resulted in catch-up growth in this model. Ovine (o)CRH testing results were compatible with a hyperactive HPA axis, as we had seen in patients with adult melancholic depression and sexually abused preadolescent girls.[2,6] Pine et al[25] recently reported a small loss of final height in young girls with childhood anxiety disorder, but not in those with childhood and adolescent depression.[25] These data are in agreement with the slightly hyperactive HPA axis in the former and the normal or hypoactive HPA axis in the latter.[13] No compromise in the final stature of boys with anxiety disorder was observed in the study by Pine et al. This may be explained by the higher estrogen levels in girls than in boys, enhancing CRH secretion.[15]

Premature infants are at risk for delayed growth and/or development, especially after prolonged hospitalization in the intensive care nursery. The condition is similar to PSS, but is known as "reactive attachment disorder of infancy" and can be prevented and/or treated with loving attention.[26] Interestingly, activation of the human fetal HPA axis is also associated with growth retardation. Elevated levels of CRH, ACTH, and cortisol have been reported in growth-retarded fetuses.[27]

Infantile and childhood malnutrition is characterized by hypercortisolism, decreased responsiveness to CRH and incomplete dexamethasone suppression, and the euthyroid sick syndrome pattern of thyroid hormone abnormalities, all of which are restored after nutritional rehabilitation. It is noteworthy that in this condition, increases rather than decreases of GH secretion are present, possibly resulting from starvation, which induces hyposecretion of IGF-1 and resultant decreased negative feedback upon GH secretion.

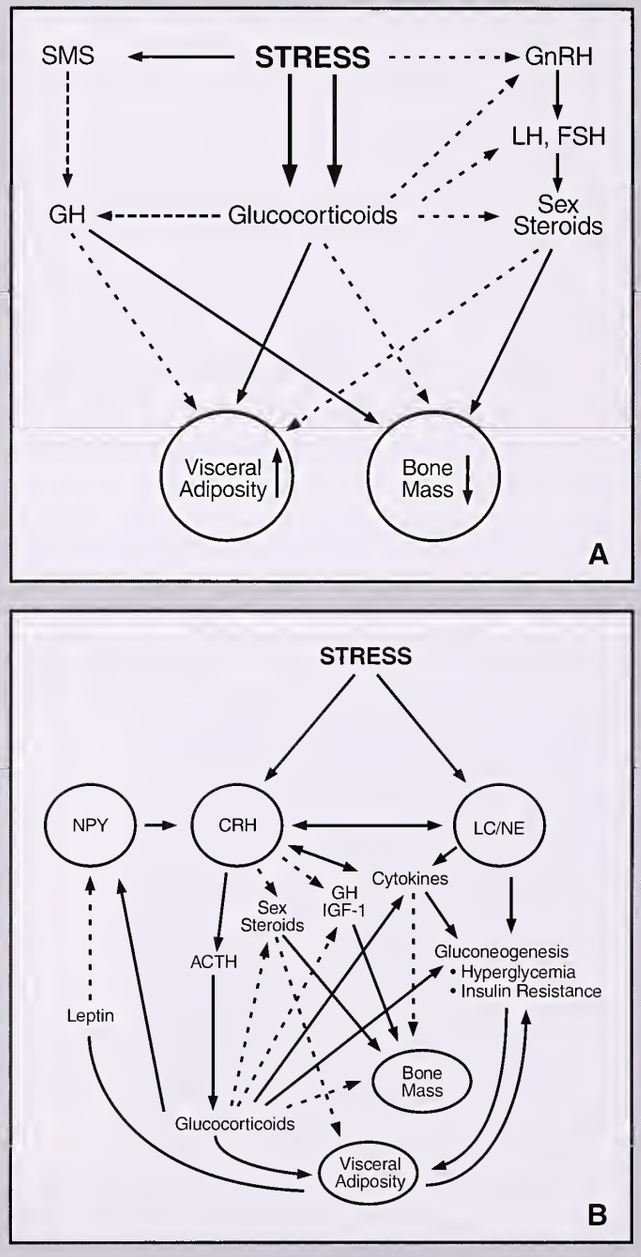
STRESS AND METABOLISM

Long-term administration of glucocorticoids or endogenous Cushing syndrome is associated with visceral obesity, insulin resistance, hypertension, and elevated cholesterol and triglyceride levels (Figure 4A). Thus, hypercortisolism resembles "metabolic syndrome X" (MS-X) in both its somatic and biochemical phenotypes. Interestingly, MS-X was recently associated with increased 24-hour urinary free cortisol excretion, suggesting that glucocorticoids may represent a common denominator of both states.[28] Moreover, both hypercortisolism and MS-X are associated with increased atherosclerosis and resultant cardiovascular morbidity and mortality.

The association between chronic, experimentally induced psychosocial stress and a hypercortisolism/MS-X–like state, with resultant marked coronary atherosclerosis, was recently reported in cynomolgus monkeys.[29] In these animals, chronic, stress-induced activation of the HPA axis and, therefore, hypercortisolism and suppressed GH secretion apparently led to visceral obesity, insulin resistance, hypertension, and dyslipidemia (Figure 4B), all converging to the development of varying degrees of the physical and biochemical phenotype of MS-X.

"Low turnover" osteoporosis is almost invariably seen in association with hypercortisolism and GH

Figure 4



A simplified schematic representation of the interactions between the stress system and metabolic functions. Adapted with permission from Chrousos and Gold.[2]

deficiency, reflecting the detrimental effect of the combination of high cortisol and low GH and/or IGF-1 concentrations on the osteoblasts. Osteoporosis may be further potentiated by the stress-related hypogonadism. We recently reported increased prevalence of "low turnover" osteoporosis associated with decreased plasma osteocalcin levels in relatively young women with depression or a history of depression.[30]

STRESS AND IMMUNE FUNCTION

Activation of the HPA axis takes place during the stress of an infectious disease, autoimmune inflammatory process, and accidental or operative trauma.[31] The mechanisms of this association have been unraveled recently. The 3 "inflammatory cytokines"—TNF-α, IL-1, and IL-6—cause stimulation of the HPA axis *in vivo*, alone or in synergy with each other (Figure 4B). This is mediated by hypothalamic CRH and AVP secretion and by direct effects at the pituitary and adrenocortical levels. IL-6, the main endocrine cytokine, causes major elevations of ACTH and cortisol, elevations well above those observed with maximal stimulatory doses of CRH, suggesting that AVP and potentially other ACTH secretagogues are also stimulated by this cytokine. Glucocorticoids and prostanoid synthesis inhibitors suppress the stimulatory effects of cytokines on the HPA axis.

Glucocorticoids, the end-hormones of the HPA axis, play a major role in the stress-induced suppression of the immune/inflammatory reaction.[31] On the other hand, the autonomic system also participates in the effects of stress on the immune/inflammatory reaction, both by being reciprocally connected with the CRH system and by transmitting neural signals from the CNS to the immune system. The latter is mediated by a dense innervation of both primary and secondary lymphoid organs, and by reaching sites of inflammation via postganglionic sympathetic neurons. The sympathetic system, when activated, causes systemic secretion of IL-6, which by directly inhibiting the other 2 inflammatory cytokines, TNF-α and IL-1, and by activating the HPA axis participates in the stress-induced suppression of the immune/inflammatory reaction. Also, catecholamines via β-adrenergic receptors inhibit IL-12 and stimulate IL-10 and IL-4 secretion and, hence, cause suppression of cellular immunity and stimulation of humoral immunity.[32]

The HPA axis and the immune system function in balance in the physiologic state.[31] An excessive response of the HPA axis to inflammatory stimuli mimics the hypercortisolemic state and leads to increased susceptibility of an individual to certain viral and bacterial infections or neoplasia. On the other hand, a defective HPA axis response to inflammatory stimuli reproduces the glucocorticoid-deficient state and leads to increased susceptibility to allergic/autoimmune/inflammatory diseases.

SUMMARY

In response to a stressor that exceeds a threshold magnitude, or multiple stressors applied simultaneously, the organism alters its behavior and physiology with the aim of maintaining homeostasis. The adaptive changes that occur are coordinated and mediated by the stress system in the CNS — which includes CRH-peptidergic and noradrenergic neurons in the hypothalamus and the brain stem, respectively — and its peripheral limbs, the HPA axis and the autonomic (sympathetic) system. Controlled or self-driven challenges to homeostasis and a normally functioning stress system are crucial for normal development and the preservation of self and species. In childhood and adolescence, during which psychosexual maturation and growth take place, appropriately functioning neuroendocrine responses to stressors are necessary to allow these processes to progress normally. Maladaptive neuroendocrine responses, ie, dysregulation of the stress system, may lead to disturbances in growth and development, and cause developmental/psychiatric, endocrine/metabolic, and/or autoimmune disorders or vulnerability to such disorders not only during childhood and adolescence but also in adulthood.

Editor's comment: *Pertinent to the content of this excellent article is the abstract elsewhere in this issue, entitled "A New Stress-Related Syndrome of Growth Failure and Hyperphagia in Children Associated With Reversibility of GH Insufficiency," and the letter to the editor from the authors.*

Robert M. Blizzard, MD

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Erratum

In the last issue of *GGH*, the starting date that Dr. Lifshitz became Chief of Staff at Miami Children's Hospital was incorrectly stated as February 1, 1977. The correct date is February 1, 1997.

Letter to the Editors

Dear Dr. Blizzard:

This letter concerns a paper by Tillotson et al (*GGH* 1996;12[2]:30). The paper contains a methodologic error and the results are invalid. However, the authors' conclusion that the mental and neurologic features of phenylketonuria (PKU) are not caused prenatally can rest on other evidence and is perfectly sound. Other workers who by luck or judgment avoid this error and get different results may tend to discard Tillotson et al's conclusion with their results. This is the reason for submitting this letter.

Since PKU is not panethnic and ethnicity affects birth weight, it is not valid to compare birth weights of affected infants with statistical norms for an entire multiethnic population such as that of the United Kingdom (*Eur J Pediatr* 1995;154(10):847-849). In investigations (*Int J Neuroscience* 1990;54:259-266) in Ireland and west Scotland, where there is a very high incidence of PKU in an ethnically homogeneous population, there was no significant difference in birth weight between PKU infants and their unaffected siblings ($P>0.5$), as others had previously found. However, closely matched control infants drawn from the same populations had mean birth weights 107 g > than the PKU infants and their unaffected siblings ($P<0.02$). A similar finding was later made in the Netherlands, although the authors did not investigate the birth weights of unaffected siblings of PKU infants (*Arch Dis Child* 1994;71[2]:114-118).

In the Irish and west Scottish investigation, the birth weights of the PKU infants and their unaffected siblings lay on a single normal distribution curve with no evidence of bimodal or trimodal distribution. Since the unaffected sibs are mentally and neurologically normal, but show a reduction in birth weight equal to that of PKU infants, the lower birth weight cannot be related to the pathogenesis of PKU or to the fetal genotype. The reduction in birth weight can be a reflection only of maternal heterozygosity and constitutes a previously unknown effect of the PKU gene in a single dose.

Respectfully submitted,

L.I. Woolf, PhD
Professor Emeritus
University of British Columbia
Vancouver, British Columbia

Editor's comment: *Dr. Tillotson was contacted twice regarding this letter from Dr. Woolf, but no response was forthcoming.*

Robert M. Blizzard, MD

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Post-Program Self-Assessment/CME Verification Answer Sheet

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A New Stress-Related Syndrome of Growth Failure and Hyperphagia in Children, Associated With Reversibility of Growth-Hormone Insufficiency

Growth failure without organic cause but associated with behavioral disturbances and psychosocial stress has been termed psychosocial short stature (PSS), reversible hyposomatotropism, the garbage can syndrome, and maternal deprivation. The authors state that PSS does not describe a valid diagnostic entity, but encompasses failure to thrive, stunting secondary to chronic malnutrition, and idiopathic hypopituitarism. Some of these children show spontaneous catch-up growth when removed from the source of stress, but until now precise definition of this subgroup for the purpose of clinical identification has not been possible.

The authors compared 31 normal children with short stature identified from an epidemiologic survey versus 51 children with growth failure unrelated to organic pathology who were referred to the hospital between 1986 and 1994. An additional subject who was community-identified was added to the hospital-referred group, raising the total number of hospital referred patients to 52 and the total sample to 83 patients. Growth hormone (GH) dynamics were studied in the hospital group of 52 patients by a combination of diurnal GH profiles and provocative tests for GH release. The tests were repeated after a hospital stay of 3 weeks away from familial stress. The mean age of referral of the stressed children was 7.9 years (range, 3.8 to 13.7 years). All but 2 were prepubertal. Nine sibling-pairs, including 2 sets of dizygotic twins, were included. Height for age was below the 3rd percentile. Only 8 of the 52 had not been emotionally, sexually, or physically abused.

In a distinctive subgroup (29 of the 52) growth hormone insufficiency (GHI) was associated with hyperphagia, polydipsia, and normal body mass index. When the children were removed from their stressful homes, GHI spontaneously resolved *only* in formerly hyperphagic subjects. A distinctive correlation was reported between GHI, hyperphagia, and growth recovery when the children were removed from the home. Seventy-four percent of the hospital-referred group (n=23) were identified as being nonhyperphagic with anorexia, low body mass index, and normal GH response to provocation tests.

The authors identified 9 key symptoms that distinguished the hyperphagia/polydipsia group (n=29) from the nonhyperphagic (n=23) and community comparison (n=31) groups (Table 1). The hyperphagic children were distinguishable from the nonhyperphagic children usually by using standard provocative tests of GH secretion. Five of the hyperphagic children were admitted for at least 3 weeks for testing of spontaneous changes in growth hormone secretion. The remaining 16 hyperphagic children had initial provocative tests of growth-hormone secretions as inpatients on the day of admission. Fourteen of these 16 hyperphagic children (88%) were found to be growth hormone insufficient initially. After about 3 weeks of restricted parental contact, 10 of the 16 had a distinctly increased GH release. Sixteen of the nonhyperphagic children were investigated by at least 1 GH provocative test. Six nonhyperphagic children (38%) had evidence of GHI on initial testing. The mean initial response was greater than in the hyperphagic children initially, 22.6 mU/L versus 8.9 mU/L. The mean of the peak GH in these nonhyperphagic children was 26.7 mU/L, when testing was repeated at 3 and 6 weeks.

Table 1
Characteristics of Appetite Disturbance

	Hyperphagic Patients (n=29)	Nonhyperphagic Hospital-Referred (n=23)	Community Comparisons (n=31)
Eats too much	25 (86%)	2 (9%)*	0*
Gorges and vomits	23 (79%)	1 (4%)*	0*
Steals food at home	28 (97%)	3 (13%)*	3 (10%)*
Steals food at school	16 (55%)	0*	0*
Hoards food	19 (66%)	2 (9%)*	1 (3%)*
Drinks excessively	17 (59%)	2 (9%)*	12 (39%)*
Pica	18 (62%)	0*	0*
Eats from bins/discarded food	16 (55%)	0*	0*
Searches for food at night	18 (62%)	1 (4%)*	0*

Analyses were planned comparisons between hyperphagic subjects and those in the other 2 groups separately. Symptoms were recorded as present if they were happening now or if they had occurred within the previous 6 months. *$P<0.001$.

Skuse D, et al. A new stress-related syndrome of growth failure and hyperphagia in children associated with reversibility of growth-hormone insufficiency. *Lancet* 1996;348:355.

In the discussion, the authors suggested that the 29 patients with hyperphagia and polydipsia constitute a previously undefined syndrome of growth failure. They propose that the condition of hyperphagic short stature has predictive and discriminant validity on the basis of its symptom profile: GHI, associated intellectual impairment, and familial aggregation. The authors state that failure to thrive during infancy occurred in 95% of the hyperphagic subjects, but also in 40% of the nonhyperphagic hospital cases. The authors suggest that nosologic confusion could be avoided if the term hyperphagic short stature is used to identify patients with hyperphagia/polydipsia, and these patients should strongly be suspected of having GHI. They suggest that in the nonhyperphagic patients (75% with anorexia), chronic nutritional deficiency is very frequently present and causes stunting.

The authors further interpret the data by stating that the explicit behavioral and developmental criteria by which the novel syndrome of hyperphagic short stature may be clinically recognized was described. Such children were stated to have a capacity for spontaneous recovery of GH production when removed from the adverse environment. Discriminant and predictive validity of the core symptoms was demonstrated and preliminary familial studies indicated a possible genetic predisposition, as there were 9 sets of sibs. including 2 pairs of dizygotic twins.

Skuse D, et al. *Lancet* 1996; 348:353-358.

Editor's comment: *The readers are referred for summary of current knowledge about PSS to Chapter 6 in the Third Edition of* Pediatric Endocrinology, *edited by Fima Lifshitz. (Marcel Dekker Publishers, 1996). The table below is a classification published in that text.*

Skuse et al are to be commended on furthering our knowledge about the variability within the group of patients described as having PSS type II. Skuse et al have added clarification to the variability of the syndrome if one uses the umbrella term of PSS for children who are short and who experience a high degree of parental psychological neglect or trauma. Undoubtedly, the data dividing hyperphagic and nonhyperphagic patients is a service and clarification. The demonstration that in type II PSS, as defined by Blizzard and Bulatovic, there may be subdivisions of hyperphagic and nonhyperphagic patients is a contribution. The fact that hyperphagic patients are those who most frequently have associated GHI is a significant contribution also.

I disagree that this can be called a "new" stress-related syndrome of growth failure and hyperphagia in children, associated with reversibility of GHI. Instead, I interpret these findings to further clarify the variable types of PSS. The need to have a valid diagnostic entity broken out of PSS may be tenable for medical administrative reasons, and that is acceptable, if necessary. However, as pointed out by Blizzard and Bulatovic in Lifshitz' text, there are different types of PSS, including failure to thrive in some cases brought on by psychological stress, and other types as described by Boulton et al (GGH *1992;8[4]:13). The latter was labeled as type III in the overall classification of PSS by Blizzard and Bulatovic. Perhaps the designation of type IIA for the PSS of the hyperphagic type and type IIB PSS for the anorexic type would be more preferable for classification purposes, until we have additional data to break out the subtypes of growth retardation that should be considered under the umbrella of PSS.*

An additional approach to the hyperphagic versus the anorexic patients looked at by Skuse et al would be to examine these patients in respect to depression. It has been pointed out previously that depression is a common finding in all PSS types. Another route of investigation for the authors would be to examine their 2 groups of affected patients in respect to their growth response to GH treatment. Type II patients as recorded by Blizzard and Bulatovic are resistant to GH treatment.

I urge that pediatric endocrinologists incorporate in their thinking the type of hyperphagic PSS described by Skuse et al as being only one type, therefore type IIA. For purposes of further study, it is better to call their hyperphagic patients a subtype of PSS at this point in time. Further study comparing type III as described by Boulton et al with the nonhyperphagic/anorexic group described by Skuse et al is desirable, as the anorexic patients described by Skuse et al may fall in type IIB, as proposed above, or in type III, which would eliminate the need to have 2 subtypes of category II. The authors of this fine paper and all others are urged to further clarify the variability of recognized PSS syndromes rather than describe new syndromes.

Robert M. Blizzard, MD

Characteristics of Various PSS Syndromes

Type	Age of Onset	Failure to Thrive	Bizarre Behavior	Depression	GH Secretion	Parental Rejection	GH Responsiveness
I	Infancy	Usually	No	Often	Normal	No	?
II	≥3 years	Some and some overweight	Usual	Very often	Decreased or absent often	Usual	Minimal at doses used
III	Infancy or later	Not usual	Not usual	Yes	Normal	Concern, not rejection	Significant at dose used

Lifshitz F. *Pediatric Endocrinology*. New York, NY: Marcel Dekker Inc; 1996:3rd Edition, Chapter 6.

Response by Authors: We agree with Professor Blizzard's comments. We wish to identify patients with potentially reversible GH secretion who could achieve catch-up growth without GH therapy. Eighty percent of our patients with hyperphagic short stature experienced occult physical, emotional, or sexual abuse. Treating such children with GH daily for many years, without recognizing child abuse, would add insult to injury.

There was no evidence of depression in our patients. GH reversibility was demonstrated in only 1 of 6 nonhyperphagic children in the hospital-referred comparison group who were not anorexic. Two patients had no reversibility of GH secretion.

We understand the point of view that a new syndrome has not been described, but rather a more specific subtype within type II classification of PSS. Nevertheless, considerable evidence of the syndrome's distinctive nature has been gathered. This includes evaluation of a new series of patients. The syndrome is not apparently on a qualitatively similar continuum with other types of PSS, as our data strongly indicate only some children are predisposed to develop it, even within a sibling group. We agree that if classified within Blizzard and Bulatovic's scheme, hyperphagic short stature will fall into category type II, or possibly type IIA. In this condition, GH deficiency reverses spontaneously with environmental manipulation, and such patients often are resistant to GH treatment. Our nonhyperphagic subjects without an eating disorder, which in our experience is rarely associated with GH deficiency, could possibly be classified as type IIB. Patients in this group only occasionally respond to GH treatment.

As induced injury can be considered as a spectrum of disorders, ranging from factitious injury to the Munchausen by Proxy syndrome, PSS also is a spectrum of morbidity ranging from environmentally induced growth failure due to chronic undernutrition to growth failure caused by the endocrinopathy originally described by Powell and colleagues in 1967. Our work has not only confirmed these findings in that hyperphagia and polydipsia [were part of the clinical picture of their cases, but we also have shown that hyperphagia and polydipsia] are very sensitive markers that suggest the patient probably has reversibility of GH deficiency. The syndrome is a remarkable example of the potential responsiveness of a neuroendocrinologic system to stress.

Our clinical impression is that many children with hyperphagic short stature are labeled as having true GH deficiency following a day-case evaluation of GH secretion. The true diagnosis is missed. We emphasize the importance of asking the appropriate questions in order to make the correct diagnosis and to reveal that occult child abuse is probable in children with the disorder. The consistency of the symptom constellation in affected children is remarkable. The physician should routinely ask whether a child with growth failure and GH deficiency eats excessively; gorges and vomits if given unlimited access to food; steals food at home and school; hoards food; has polydipsia or pica; scavenges from trash cans; or searches for food at night. We wish to emphasize that among children with GH deficiency, the syndrome of hyperphagic short stature is not as rare as previously believed.

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Holoprosencephaly and Sonic Hedgehog

The "hedgehog" story is emerging rapidly. The hedgehogs are a family of developmentally important signaling proteins first discovered in the fruit fly, *Drosophila*. There are 3 human hedgehog genes known to date: *Sonic Hedgehog* (*SHH*), *Indian Hedgehog (IHH)*, and *Desert Hedgehog (DHH)*. They encode secreted proteins that undergo autocatalytic cleavage to produce a carboxy-terminal fragment and a biologically active amino-terminal fragment that tends to remain near the cell of origin.

The hedgehog proteins have been shown to have effects on the developing embryo in many species, including patterning effects on the midline central nervous system (CNS) structures and on developing limbs. Indeed, genetic inactivation of *SHH* in mice produced cyclopia and other CNS abnormalities, raising the possibility that mutations of *SHH* could be responsible for some human birth defects involving these structures.

These latter observations prompted groups headed by Muenke and Tsui to consider *SHH* as a candidate for alobar holoprosencephaly type 3 (HPE3), which behaves as a dominant trait with wide clinical variability in some families. It had been previously mapped to chromosome 7q36. This form of holoprosencephaly involves failure of the forebrain to divide into right and left hemispheres. At the severe end of the spectrum, it is typically associated with midline facial abnormalities, including cyclopia, a primitive nasal structure (proboscis), and clefting. Manifestations at the mild end of the spectrum may be limited to microcephaly, mild hypotelorism, midline facial clefts, and a single maxillary central incisor.

In the first paper, Belloni et al defined a critical region of about 500 kb for *HPE3*. This was done from physical mapping of breakpoints for chromosomal rearrangements in several HPE3 patients. Next, they mapped *SHH* to this interval. Interestingly, none of the breakpoints disrupted *SHH*.

From analysis of *SHH* in 30 families, Roessler et al subsequently detected heterozygous mutations, which segregated with *HEP3* phenotype, in 5 families. Two mutations were nonsense mutations predicted to cause premature termination of the *SHH* protein. Another predicted disruption of the autocatalytic cleavage site. All 3 would be expected to produce loss of function of one of the *SHH* alleles, haploinsufficiency. The paper, as well as invited comments, speculated about how such mutations could cause such profound effects on craniofacial development.

Belloni E, et al. Identification of *Sonic hedgehog* as a candidate gene responsible for holoprosencephaly. *Nature Genet* 1996;13: 353-356. Letter.

Dean M. Polarity, proliferation and the *hedgehog* pathway. *Nature Genet* 1996;14:245-247. News and Views.

Roessler E, et al. Mutations in the human *Sonic Hedgehog* gene cause holoprosencephaly. *Nature Genet* 1996;13:357-360. Letter.

***Editor's comment**: Signaling through the hedgehog family of proteins is becoming very interesting. In the past year,* IHH *has been implicated in a negative feedback loop controlling the rate of endochondral bone growth. Mutations of the hedgehog receptor,* patched, *have been found in the Gorlin syndrome and in sporadic basal cell carcinoma; and now mutations of* SHH *appear to cause some forms of holoprosencephaly. Given the apparent importance of hedgehog signaling in so many regulatory circuits, one wonders how many other sporadic disorders, especially those involved in craniofacial and limb development, might also be due to defects in hedgehog signaling.*

William A. Horton, MD

Constitutively Activated Receptors for Parathyroid Hormone and Parathyroid Hormone-Related Peptide in Jansen's Metaphyseal Chondrodysplasia

Jansen's metaphyseal chondrodysplasia is a rare form of short-limbed dwarfism associated with hypercalcemia and normal or low serum concentrations of parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrP). It is an autosomal dominant genetic disorder. Most cases are due to new mutations. Jansen's metaphyseal chondrodysplasia is recognized in the newborn period by rhizomelic short stature, severe bowing of the legs, fronto-orbital asymmetry, hypertelorism, and hypoplasia of the mandible. X-ray films show cupping and irregularity of the growth plates. All metaphyses are severely involved and appear markedly enlarged, wide, irregular, and cystic. Laboratory findings include increased serum calcium and alkaline phosphatase, with normal or low PTH and PTHrP.

The actions of both PTH and PTH-related hormones are mediated through PTH-PTHrP receptors, and their intracellular signaling is mediated by both cyclic AMP (cAMP) and calcium. PTH-PTHrP receptors belong to the family of G protein-coupled receptors, which have dual signaling properties. They are expressed in many fetal and adult tissues and found in abundance in kidney, bone, and growth-plate cartilage.

Schipani et al have confirmed the presence of a mutation in the gene for PTH-PTHrP receptors in 4 of 6 additional individuals with Jansen's metaphyseal chondrodysplasia. A similar mutation has previously been identified by Schipani et al in an individual with Jansen's metaphyseal chondrodysplasia.

Three of the mutations found had the histidine changed to arginine at position 223 (H223R). One had a novel missense mutation that changed a threonine in the receptor's sixth membrane-spanning region to proline (T410P). None of these mutations were found in the healthy relatives. In one family, the H223R mutation was found in the affected mother and her affected daughter but not in the healthy father.

Mutations cause activation of PTH-PTHrP receptors, resulting in hypercalcemia and hypophosphatemia resembling that of humoral hypercalcemia of malignancy seen in some breast cancer tissues and some hematologic cancers such as adult T-cell leukemia. The mutant receptor seems to be constitutively active in Jansen's metaphyseal chondrodysplasia and its actions appear to be independent of PTH and PTHrP. When the authors compared the PTH and PTHrP receptors containing the H223R mutation with those containing the T410P mutation, they found that receptors containing the T410P mutation had significantly higher ligand-stimulated accumulation of AMP and inositol phosphate and that receptor activation (receptor function) was independent of PTH and PTHrP. Although there were differences in receptor functions between the 2 types of mutations, the manifestations of the disease were similar with both types of mutations in affected individuals. The 2 individuals without identifiable mutations had somewhat milder disease, with less severe hypocalcemia, normal serum phosphorus and alkaline phosphatase activity, normal serum PTH concentrations, and normal urinary cAMP excretion.

Schipani E, et al. *N Engl J Med* 1996;335:708-714.

***Editor's comment**: The discovery of this constitutively activating mutation has brought to light yet another physiologically important role of PTHrP in fetal bone growth and cellular differentiation. With recent advances in molecular biology as well as in tissue engineering, perhaps it may be possible to correct the abnormality during embryonic and fetal development by in utero gene manipulation and thus eliminate the disorder. This also may be true for some other activating receptors in disorders of growth and other metaphyseal dysplasias.*

Judith G. Hall, MD

A Placebo-Controlled, Double-Blind Trial of Growth Hormone (GH) Treatment in Prepubertal Children After Renal Transplant

Although it has been shown that GH therapy can increase growth velocity (GV) in children with chronic renal failure, similar data have not been shown in prepubertal children following renal transplantation. The initial increase in growth rate following transplantation declines such that up to 70% of prepubertal children do not show significant catch-up growth in the first 2 years after transplantation. The most likely cause of this is prolonged immunosuppressive therapy.

Hokken-Koelega et al, used a double-blinded, placebo-controlled, 6-month cross-over trial of biosynthetic human GH (hGH) in 11 prepubertal patients (9 boys and 2 girls) post renal transplantation. Inclusion criteria included (1) at least 12 months post transplantation; (2) no rejection episodes within the last 6 months; (3) height standard deviation score (SDS) for chronologic age below -1.88; (4) height velocity (HV) for chronologic age below the 50th percentile or a height SDS for chronologic age above -1.88 with a HV below the 25th percentile; (5) prepubertal; (6) bone age (BA) < 10 years for girls and < 12 years for boys; (7) prednisone dose not exceeding 0.25 mg/kg/d; (8) normal thyroid function studies; (9) normal acid-base balance; (10) no previous treatment with sex steroids; and (11) no evidence of specific reasons for growth retardation other than renal transplantation.

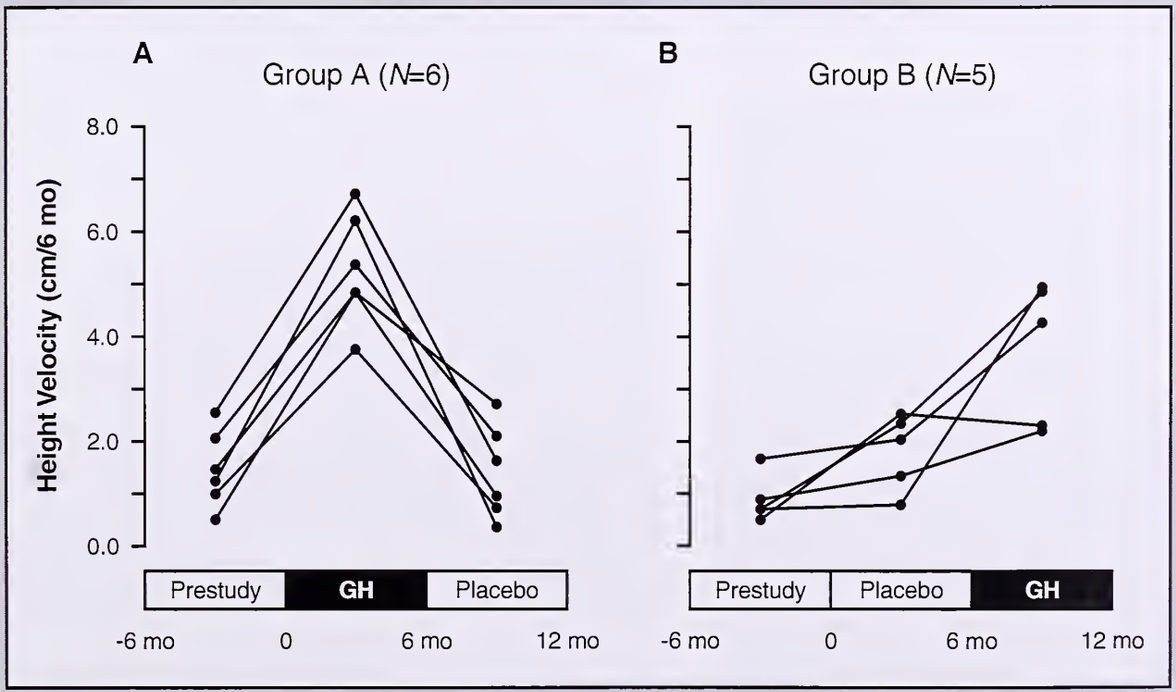
Subjects had a mean age of 12.1 ± 2.9 years, with a range of 8 to 18 years. Immunosuppressive therapy consisted of prednisone with either azathioprine or cyclosporine, or a combination of both. Children were randomly and blindly assigned to receive 1 subcutaneous injection a day of either biosynthetic hGH (4 IU/m^2, roughly equal to 0.05 mg/kg) or an equal volume of reconstituted placebo. Patients were measured and weighed every 3 months. Mean height was expressed as SDS for CA, as was HV. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), glucose, and oral glucose tolerance were determined at 0, 6, and 12 months, and insulin-like growth factor 1 (IGF-1), IGF-2, IGF-binding protein 1 (IGFBP-1), and IGFBP-3 were measured.

Children on biosynthetic hGH therapy increased their HV significantly more than those receiving the placebo: 5.3 ± 1.0 cm per 6 months versus 1.9 ± 0.7 cm per 6 months; $p<0.0001$ (see Figure 1). The mean increase in HV during biosynthetic hGH treatment was significantly higher for those who started the trial with biosynthetic hGH than for those who started with placebo. No significant increase in bone maturation occurred during the study. Mean IGF-1 increased significantly while the concentration of IGF-2 and IGFBP-3 did not. No patient had an acute rejection episode, and there were no differences in GFR or ERPF between the 2 groups.

The authors state that their data show that synthetic hGH therapy results in an improvement in HV in prepubertal children with growth retardation after renal transplantation. Long-term studies are necessary in order to determine whether the effect will be sustained and whether there will be significant improvement in final height.

Hokken-Koelega A, et al. *Kidney Int* 1996;49(suppl 53):S128-S134.

Figure 1



Individual height velocities in 11 patients post renal transplantation.

Editor's comment: *This is an important study but the data should be viewed as preliminary. The lack of significant changes in plasma IGF-2 and IGFBP-3 may have been due to the relatively small number of individuals studied since trends were evident. In addition, the short length of the study may have contributed to the lack of failure to demonstrate any significant changes in skeletal maturation secondary to biosynthetic hGH therapy. Finally, it may not be appropriate to report HV for CA when the subject population includes individuals as old as 18 years who are prepubertal. Despite these shortcomings, the authors should be commended for utilizing a placebo-controlled trial of biosynthetic hGH in prepubertal children. Their data are encouraging, and we look forward to further data as they continue to use biosynthetic hGH therapy in these children.*

The reader is referred to the lead article by Fine in GGH *(1996;12[4]:49-53) regarding the use of biosynthetic hGH over several years in patients with chronic renal failure.*

William L. Clarke, MD

Serum Leptin in Children With Obesity: Relationship to Gender and Development

The aim of this study was to investigate whether leptin can be detected in the serum of obese children; to determine whether it directly correlates with body fat, as it does in adults; and to identify any variations of leptin concentration in relation to gender, race, and growth and development. All values were expressed as mean ± SD.

Seventy-seven children (44 girls and 33 boys; mean age 11.3 ± 4 years) with obesity (body mass index [BMI]) > 95th percentile; mean BMI 34.4 ± 7.6 kg/m^2), and 30 normal weight children (20 girls and 10 boys; mean age 13.3 ± 3.5 years and mean BMI 18.9 ± 3.1 kg/m^2) were studied.

Serum leptin was measured by radioimmunoassay in all study subjects in a blood sample obtained after an overnight fast. Children in the control group were slightly older than the obese children but differences in Tanner staging were not significant. Serum leptin in the obese group was significantly higher than that in the control group (38.6 ± 21 ng/mL vs 7.8 ± 6.5 ng/mL). All children had detectable leptin concentrations. The range of leptin concentrations in the obese group was 4.9 to 84.6 ng/mL. Serum leptin directly correlated with BMI and upper-arm fat area analysis in the combined (obese and control) group (r=0.88, P<0.001 and r=0.88, P<0.001, respectively). This is similar to the correlation described in adults. A mild direct correlation (r=0.51, P<0.001) was found between serum leptin and fasting insulin levels. Girls demonstrated higher serum leptin levels than boys. The effects of sexual development on leptin concentration were also positive. Subjects with advanced Tanner stages displayed lower leptin levels than earlier Tanner stages, independently of adiposity.

There were no effects detected among different races independent of the estimate of body fat. The authors conclude that, as in adults, obese children have high concentrations of leptin, which directly correlates with arm fat and BMI. They hypothesize that a relative central "leptin resistance" is part of the normal process of growth and development during childhood.

Hassink SG, et al. *Pediatrics* 1996;98:201-203.

Editor's comment*: A new world order for pediatric endocrinologists is now upon us. The fat tissue is the source of leptin. This paper is only the beginning of what will be forthcoming in this area. The authors' interpretation of the inverse relation between leptin levels and the degree of sexual maturation independent of the adiposity leads to the hypothesis that children display a relative central "leptin resistance,"*

In Future Issues

Tyrosine Kinases and Cancer
Brian Druker, MD

GH Secretagogues
Allen Root, MD

Insulin, the IGF System, and Insulin-Dependent Diabetes Mellitus
Cheri Deal, MD

The Pathophysiology of Growth Failure in Renal Disease: An Update
David Powell, MD

Genetic Basis of Human Chondrodysplasias: A Review
William Horton, MD

which, if true, would favor an increased energy intake necessary for growth and development. A switch to an increased leptin sensitivity would occur at the end of puberty, signaling the end of growth. Even though the finding of lower leptin levels at more advanced pubertal stages found in this study is appealing to formulate that hypothesis, more direct evidence of such a relation is necessary. The perpetuation of this "physiologic" leptin resistance beyond the time of full pubertal development as a result of a disturbance in the switch to normal leptin sensitivity could be a theoretical cause for the development of obesity in early adulthood. However, this would not explain the cases of childhood obesity.

The greater levels of leptin in girls than boys, independent of adiposity, led to the hypothesis that central leptin resistance in girls may be necessary for the accumulation of the adipose tissue stores necessary for reproduction. This is an attractive thought, but the evidence provided to support this hypothesis is very indirect.

The study of leptin levels in children will provide clues on the interactions between adipose tissue and the endocrine system in the developing individual. Note that 2 articles concerning leptin recently were abstracted in GGH *(1996;12[2]: 31-32). Readers may wish to refer to these abstracts.*

Fima Lifshitz, MD

Growth Hormone (GH) Replacement in Healthy Older Men Improves Body Composition But Not Functional Ability

The objective of the study was to determine whether GH replacement in older men improves functional ability. The design was to perform a randomized, controlled, double-blind study utilizing 52 healthy men >69 years old with well-preserved functional ability but low baseline insulin-like growth factor 1 (IGF-1) levels. Recombinant human GH (rhGH; 0.03 mg/kg of body weight) or placebo was given 3 times a week for 6 months. Body composition, knee and hand grip muscle strength, systemic endurance, and cognitive function were measured.

At 6 months, lean mass increased on average by 4.3% and fat mass decreased by an average of 13.1% in the rhGH-treated group, in contrast to slightly decreased percentages in the placebo-treated group. No statistically or clinically significant differences occurred in knee or hand grip muscle strength, physical performance, systemic endurance, or cognitive function or mood as measured by the Geriatric Depression Scale, the Mini-Mental Status Examination, the Digit Symbol Substitution Test, and the Trails B Test, which is a neuropsychological test battery assessing visual and motor tracking and attention.

The dose of rhGH was considered physiologic replacement for young adults, and IGF-1 levels were maintained between 190 and 350 ng/mL by adjusting the doses. A dose decrease was necessary in 26% of the rhGH-treated group versus 0% of the control group because of side effects, which usually developed in the first month of treatment. The most common side effects were pitting lower extremity edema and diffuse arthralgias.

The authors conclude that the data do not support the hypothesis that an age-related decline in GH secretion is responsible for the functional decline of aging. Because of the lack of demonstrable efficacy in the study sample, coupled with the frequent side effects and substantial expense, rhGH should not be used to preserve or improve functional ability in healthy, functionally intact older men.

Papadakis MA, et al. *Ann Intern Med* 1996;124:708-716.

Editor's comment: *Some readers may argue that the use of rhGH in the elderly does not deal with growth or genetics, and I would agree they are correct. However, the use of rhGH in the elderly deals with the possible use of hormones to prevent or reverse aging, and is of enough significant interest to endocrinologists, particularly older ones, to prompt publication of this abstract.*

*Rudman et al (*N Engl J Med *1990;323:1-6) also reported an increase in lean body mass and a decrease in fat mass when rhGH was given to aging but otherwise normal men. Appearances before the media by aging study subjects and participating investigators conveyed the impression that rhGH was a positive force on endurance, energy, and sense of well-being. The studies reported in 1996 by Papadakis et al do not support the impressions conveyed by the media regarding Rudman et al's studies. As a proband in a similar study in (1982 through 1985), along with 4 other aging men, I can add from my own experience and from conducting body composition studies on the other 4 men that the changes in both physique and psyche were equivocal at best. Therefore, I concur with the conclusions of Papadakis et al: "GH should not be used to preserve or improve functional ability of aging men." Further studies may prove otherwise but the burden of contradicting this conclusion remains a heavy one.*

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Post-Program Self-Assessment/CME Verification
Post Self-Assessment Test Questions

Instructions: The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The CNS element that alters sensitivity to pain and may influence the emotional tone of an individual is:
   a. the amygdala/hippocampus complex.
   b. the arcuate nucleus opioid peptide-secreting neurons.
   c. the mesocortical dopamine system.
   d. the mesolimbic dopamine system.

2. Stress-induced melancholic depression is characterized by all but which one of the following symptoms:
   a. anorexia
   b. hypotension
   c. loss of libido
   d. anxiety
   e. tachycardia

3. Chronic activation of the HPA axis and the sympathetic system:
   a. causes melancholic depression in children and adolescents.
   b. may cause seasonal affective disorder and postpartum depression.
   c. is implicated as a cause of anorexia nervosa, malnutrition, and panic disorder in young adults.
   d. is evident in patients with chronic fatigue and fibromyalgia syndromes.
   e. all of the above

4. Choose the correct answer:
   a. Chronic HPA axis activation has little effect on gonadal function in highly trained athletes.
   b. CRH and the HPA axis are potential mediators of gender-related responses to stress.
   c. Lower concentrations of thyrotropin and T_3 hormones result from excessive and prolonged activity of the stress system.
   d. b and c above
   e. All of the above

5. In infantile and childhood malnutrition, GH secretion is:
   a. increased, inducing hyposecretion of IGF-1.
   b. decreased, possibly resulting from starvation.
   c. about the same as in well-nourished patients.

Answer Key: 1. B 2. B 3. C 4. D 5. A

Disclosure: As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Dr. Chrousos reports no conflicts; Dr. Lifshitz reports no conflicts; Dr. Clarke reports no conflicts; Dr. Horton reports no conflicts; Dr. Root serves on Genentech's National Cooperative Growth Study (NCGS) Advisory Committee; Dr. Hall reports no conflicts; Dr. Blizzard is the President of The Genentech Foundation for Growth and Development which functions independently of Genentech, Inc.

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Limb Lengthening in the Skeletal Dysplasias and Short Stature Conditions: State of the Art in 1997

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INTRODUCTION

Limb lengthening was first described in the Western literature in 1905 by Codivilla.[1] Until relatively recently, the indications for limb lengthening were largely confined to leg length discrepancies due to congenital or acquired conditions.

HISTORICAL ASPECTS

Limb lengthening as a technique has evolved over the last 8 decades and has involved a variety of external distraction methods. Until the introduction of the Ilizarov technique, limb lengthening was achieved by osteotomy and relatively rapid distraction of the bone ends (1 to 2 mm daily).[2] A gap resulted, usually requiring bone grafting and plate application. The most popular of these techniques, the Wagner method, was widely used in North America.[3] Numerous publications outlined complications such as hypertension, joint displacement or stiffness, compartment syndrome, and nerve palsy. Bone problems, including delayed union or nonunion and osteomyelitis, were common. Furthermore, patients required a third operation for plate and screw removal and the prolonged hospitalization of these patients often had significant psychosocial ramifications.

As Wagner popularized his techniques, a Siberian surgeon, Gavriil Ilizarov, was developing a new

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

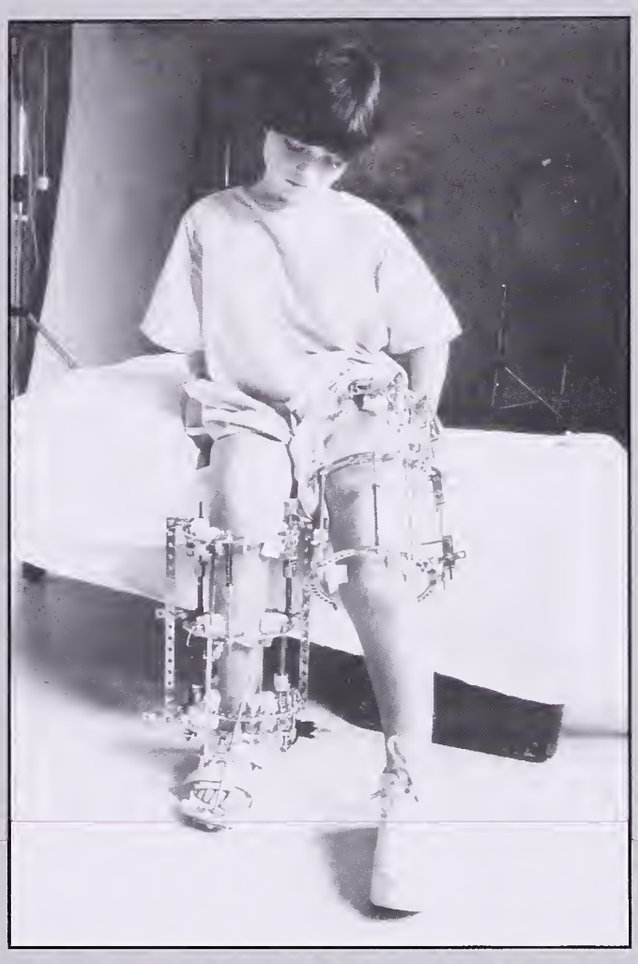
Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

In This Issue

This educational activity is supported in part by an unrestricted educational grant from Genentech, Inc.

Figure 1



Clinical appearance during initial left femoral and right tibial Ilizarov lengthening.

biology of limb lengthening.[4] As with prior methods, the technique involved the use of an external fixator. This fixator of the bones (Figure 1) differed from previous devices. It was composed of metal rings, and stability was achieved via tensioned transosseous wires. The bone was cut through a small (~1 cm) incision, with care being taken to avoid disruption of the periosteal blood supply. Gradual incremental distraction of no more than 1.0 mm daily in 3 to 4 increments resulted in new bone formation in the developing distraction gap. Once the desired bone length was achieved, the fixator was left in place until the bone consolidated. The total treatment time was roughly 1 month per centimeter of length gained. Generally, no immobilization was used following fixator removal. As opposed to the other methods, this technique encouraged weight-bearing and patient activity during treatment, thus promoting more rapid bone healing and patient rehabilitation.

The advent of this new incremental method of bone lengthening preserved bone biology, thus eliminating the need for bone grafting and plate application, and bone complications were significantly reduced. Other surgeons in Europe, North America, and Great Britain have devised other types of external fixators.[5,6] Regardless of the device, however, the current state of the art of limb lengthening involves gradual incremental distraction of the bone ends to allow new bone formation in the evolving gap.

COMPLICATIONS OF LIMB LENGTHENING

Early enthusiasm for the Ilizarov technique after its introduction to North America in 1986 saw many surgeons avidly applying the technique to an expanded list of pathologies, including short stature conditions. As with many new techniques, this early enthusiasm has been tempered by the realization that a multitude of potential complications remain.

Despite the improvement in bone healing, the Achilles heel of limb lengthening continues to be the effects on the soft tissue envelope surrounding the bone and the adjacent joints. Injuries to muscle and adjacent joints due to distraction have been described in both clinical and experimental settings.[7] Joint stiffness due to intra-articular cartilage injury and joint dislocation or subluxations are still problematic despite controlled gradual bone distraction.

LIMB LENGTH INEQUALITY

From an orthopedic perspective, the most common indication for limb lengthening is the patient with an actual or predicted leg length inequality of ≥ 5 cm. Smaller discrepancies and patients without limb deformity and normal stature are still most safely treated by appropriately timed epiphysiodesis or by femoral shortening.

Limb length inequality and limb deformity are common in the skeletal dysplasias.[8,9] The most common dysplasias causing inequality of limb length are fibrous dysplasia and Ollier disease. Bone formation occurs quite readily in these conditions, and bone in the lengthened limb ultimately mimics the quality of the bone in the original area of osteotomy. Conradi-Hünermann chondrodysplasia punctata also can be associated with limb length inequality. Successful limb lengthening has been reported in this condition and in Sillence types I and IV osteogenesis imperfecta, although this should be undertaken cautiously in the latter due to the abnormal fragility of the bone and slow bone formation. Neurofibromatosis is commonly associated with limb length inequality. Surgery should be approached cautiously in these patients as the short limb may have very abnormal bone, such as in congenital pseudarthrosis of the tibia, which will not heal predictably or remain healed.

Patients with congenital limb hypoplasia syndromes can be appropriate candidates for limb lengthening, provided that the adjacent joint stability and function can be maintained and that the foot is a suitable plantigrade weight-bearing surface. Femoral hypoplasia includes the spectrum from mild shortening to subtotal absence of the femur. Femoral hypoplasia and less severe forms of proximal femoral focal deficiency, in which the hip is stable or can be made stable surgically, are amenable to one lengthening or a series of lengthenings as indicated by the extent of the predicted limb length inequality. All of these disorders have intrinsic knee instability that can be managed if lengthening is done carefully.

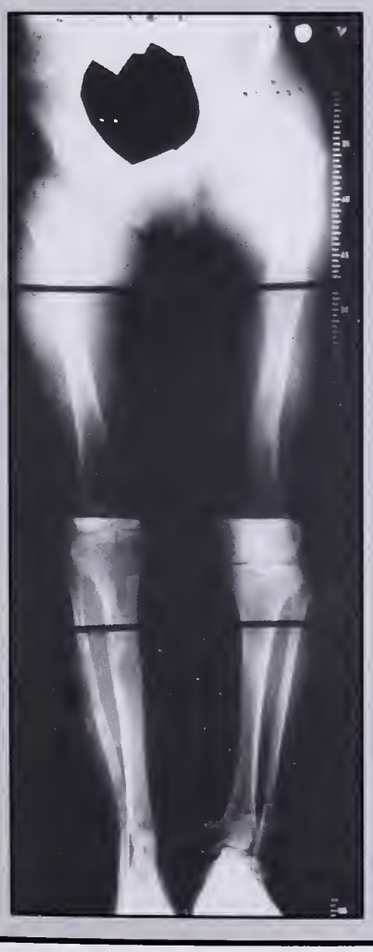
Distal deficiency, particularly fibular hemimelia, also can be managed by limb lengthening. Foot abnormality often accompanies this condition. However, if there are 3 or more rays in the foot, a reasonable result can be anticipated. The milder forms of tibial hypoplasia may also be manageable by limb lengthening. Due to the rarity of this condition, there are currently no reported series of limb lengthenings for tibial hemimelia. Severe fibular hemimelia and tibial aplasia are still best managed by amputation.

LIMB LENGTHENING IN SHORT STATURE

The first reference to limb lengthening for short stature was by Bier in 1923.[10] Since that time it has gained significant popularity in Europe, particularly Italy and Spain. The effects of short stature on psychological development are well documented.[11-13] A number of issues must be considered prior to undertaking limb lengthening. These include: the diagnosis; which limb segments are involved; and how much should the leg be lengthened and when. One of the most important factors to consider from an outcome perspective is the natural history of each disorder.[14] The epiphyseal dysplasias such as pseudoachondroplasia and spondyloepiphyseal and multiple epiphyseal dysplasia all characteristically have a natural history of early degenerative joint disease. Thus, a procedure such as limb lengthening, which may negatively impact on joint function, must be undertaken cautiously to avoid precipitating early osteoarthritis.[15]

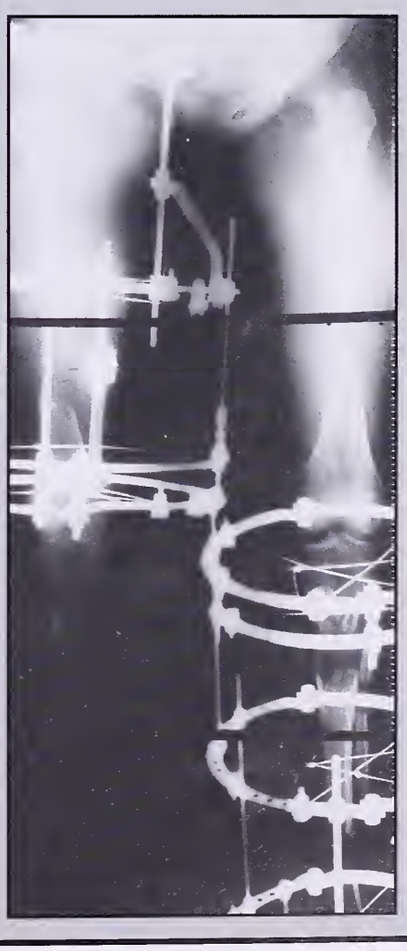
Patients with metaphyseal dysplasias such as the Schmid and McKusick types of chondrometaphyseal dysplasia may be appropriate candidates for lengthening. However, there are no reported series involving lengthening specifically in patients

Figure 2



Radiographs at the conclusion of stage 1 (7 months postoperative), with 10 cm of right tibial and 8 cm of left femoral elongation.

Figure 3



Radiographs taken 6 weeks following initiation of stage 2, right femoral and left tibial lengthening.

with these syndromes or those with epiphyseal dysplasia. Patients with Turner syndrome who have normal intelligence also represent good candidates for lengthening. Again, there are no published and very little accumulated data in this group of patients.

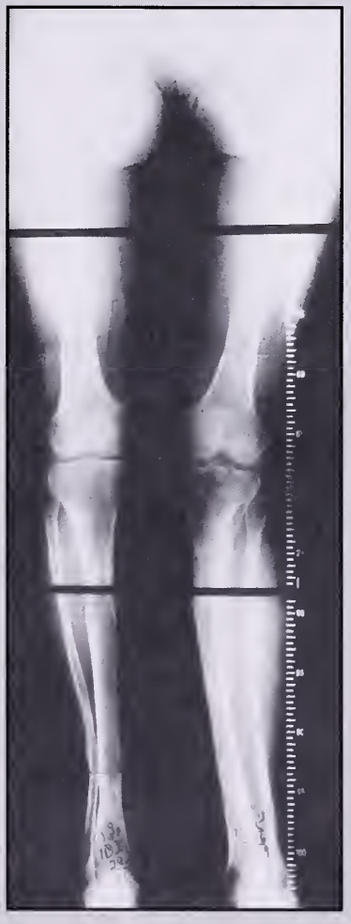
Patients with achondroplasia and hypochondroplasia are probably the ideal candidates for limb lengthening (Figures 1 through 5). Their joints are intrinsically normal and they have significant trunk-limb disproportion. One of the first English language publications concerning lengthening for short stature was by Saleh et al in Sheffield, England.[14] They reported surgery on 28 patients, 17 of whom had achondroplasia. Three had hypochondroplasia and the other 8 had other disorders. Ninety-four lower limb segments were lengthened an average of 9.6 cm. A variety of techniques were used, including the Ilizarov technique. The overall complication rate per limb segment was 71%. Of these complications, 49% were considered to be of moderate severity and the other 51% of less severe degree. Therefore, 1 out of 3 patients had moderate but significant complications.

Cattaneo et al[16] reported humeral lengthening in 29 patients, aged 10 to 36 years. Of these humeral lengthenings, 14 were in patients with achondroplasia. Total arm lengthening averaged 9 cm. All final results were excellent or good, with few complications. This currently remains the only English reference known to this author discussing elongation of the upper extremities in short stature conditions. This author's personal experience has been extremely favorable in lengthening the humeri of such patients, with complications limited to superficial pin site infection and, in one instance, pin loosening.

The results of Cattaneo et al's series of 37 lower extremity lengthenings in achondroplasia were also quite favorable.[6] Increases of 14 to 18 cm were achieved in patients who underwent femoral and tibial lengthening. The paper reported few serious complications, although critical review of ultimate functional outcome was lacking. The most significant reported complications were deformation of the bone after fixator removal; bowing and valgus deformity of the tibia; and equinus contractures at the ankle.

Both the Vilarrubias technique of limb lengthening for achondroplasia and the DeBastiani technique have gained wide popularity in Europe.[5,17,18] Both techniques utilize uniplanar external fixators. The former, however, stresses non–weight-bearing wheelchair existence during treatment. Although

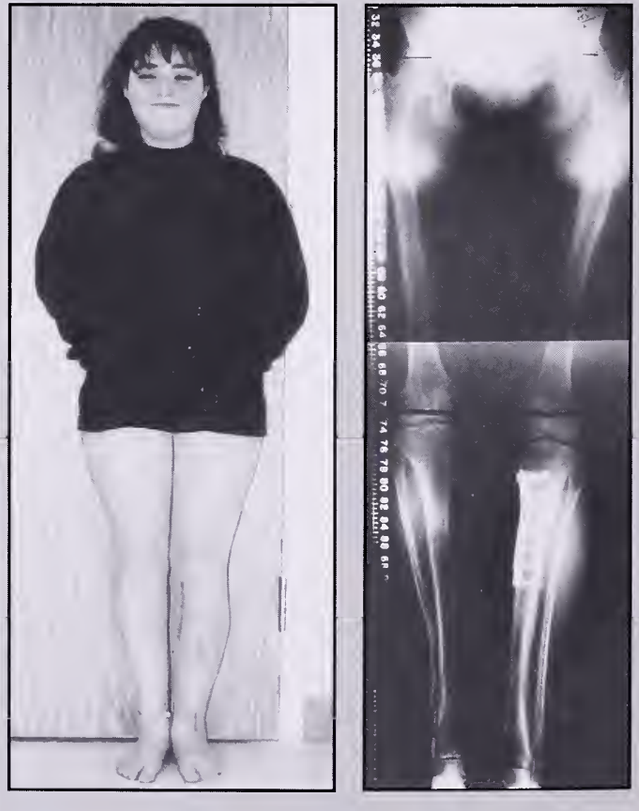
Figure 4



A left knee flexion contracture obscured mild valgus deformity of the left tibia during lengthening that was cosmetically unacceptable to the patient.

Figure 5

A B



Clinical and radiographic appearance following left tibial osteotomy at the final height of 5 ft 1 in.

results have reportedly been excellent, this encourages loss of function and independence. In North America, Price[19] has reported stature lengthening in achondroplasia using the DeBastiani technique in carefully selected patients, achieving good results and a mean height increase of 15.4 cm.[19]

A recent report[20] in 1995 discussed staged lengthening in the prevention of dwarfism in children. These authors utilized 2 separate operations on the tibiae at the ages of 5 and 10 years and 2 on the femora at 6 and 12 years. Most surgeons previously have operated primarily on older children and adults when doing bilateral lengthening for short stature. Six children reportedly had completed the first 3 stages with a total increase in length of 18 to 23 cm. This was a preliminary report and a follow-up report in a few years will be very helpful.

Issues that have not yet been adequately addressed with respect to lengthening in short stature are the ultimate functional outcomes and the effect of lengthening on limb growth. Large lengthenings put significant stress on both adjacent joints and, when open, the adjacent physes because of the increased soft tissue tension developing during distraction. The potential negative effects on joints have been discussed. Whether this stress can in fact inhibit physeal growth remains controversial in the human situation. In experimental animals, various effects, including growth stimulation and inhibition, have been reported. Maintenance or improvement in function is the goal of most surgical interventions. Limb lengthening should be no exception. However, to date, there are no adequate functional outcome studies related to limb lengthening in patients with short stature conditions.

The specific strategy adopted for limb lengthening in patients with short stature must be directed toward the goals for each individual. Ultimate height and limb proportion must be considered before developing a scheme. Patients with mesomelic dysplasia often elect only bilateral tibial lengthening, as 8 to 10 cm of length can be achieved with normalization of lower limb proportions. This strategy also may be used for patients with hypochondroplasia or Turner syndrome who require only 3 to 4 inches of height gain.

In those patients who elect more substantial lengthenings involving femora, tibiae, and, perhaps, humeri, there are several possible strategies. Bilateral tibial lengthening using either circular or cantilever fixation is generally well tolerated. This can be followed by 1 femoral lengthening and then by lengthening of the contralateral femur. In general, bilateral femoral lengthening is impossible if circular fixation is used. Occasionally, modest bilateral femoral lengthenings can be achieved using cantilever fixation. It is possible to do "crossed" lengthenings with either type of fixation. Adapting this scheme (see the case history below), 1 femur and the contralateral tibia are lengthened with either type of fixation. Eventually, bilateral humeral lengthening, which is very well tolerated, is performed. However, humeral lengthening should not be done during lower extremity lengthenings, since crutch or walker use is impossible.

Editor's comment: *The policy of* GROWTH, Genetics, & Hormones *is to not call attention to texts or similar publications, which might be interpreted as advertising. However, because there is very limited information published in English regarding limb lengthening, your attention is called to one publication that is important reading for those who are interested. This is* Limb Lengthening: For Whom, When & How? *edited by Z. Laron, S. Mastragostino, and C. Romano and published by Freund Publishing House Ltd, London-Tel Aviv. The content consists of presentations by experts on limb lengthening.* GROWTH, Genetics, & Hormones *will make this book available for a 1-week period. If the book is not mail-stamped for return within 10 days of receipt, there will be a $50.00 charge for each week the book is overdue. Requests should be made with Ms. Juanita Bishop or staff at the Genentech Foundation for Growth and Development (telephone 804-977-8192; fax 804-977-9450).*

Robert M. Blizzard, MD
Editor-in-Chief

Case History

The patient depicted in Figures 1 through 5 is a skeletally mature 14-year-old girl with hypochondroplasia. Her preoperative height at 14 years was 4 ft 5 in. Figure 1 shows her clinical appearance during initial crossed lengthenings of the left femur and right tibia using the Ilizarov technique with circular fixation. The first stage of lengthening, including distraction and consolidation, took approximately 7 months to complete. Figure 2 consists of radiographs taken at the conclusion of her first lengthening, with 10 cm of tibial and 8 cm of femoral elongation. Six months later, she embarked on her second crossed lengthenings of the right femur and left tibia. Figure 3 consists of a radiograph taken 6 weeks after initiation of these lengthenings.

A left knee flexion contracture developed during lengthening, obscuring a mild valgus deformation of the left tibia that developed during lengthening. This led to the deformity noted in Figure 4, which was cosmetically unacceptable to the patient. She subsequently underwent a left tibial osteotomy with elimination of the deformity. Figure 5 shows the final clinical and radiographic appearance. It is now 8 years since the completion of treatment.

DISCUSSION

The indications for limb lengthening in limb length inequality are clear. The indications for limb lengthening in short stature conditions remain controversial. The decision must be based on both psychological and physical factors. As discussed by Peretti,[20] Saleh,[14] and others, a lengthening "plan" must be developed. This includes the decision concerning which segments to lengthen and how the lengthening will be staged.[20] Epiphyseal dysplasias should be approached with extreme caution. However, individuals with short stature should have the choice of what are now well-established techniques in order to enhance appearance and improve function. The decision of when to perform lengthening procedures should involve the patient, not just the physician, surgeon, or parent. The author's approach to those patients seeking limb lengthening for short stature has been a team effort, involving the services of social workers, psychologists, physical therapists and consultations with other patients and families who have undergone the procedure.

Despite the technical advances in limb lengthening in North America over the last decade, there appears to be only moderate enthusiasm for limb lengthening in conditions with short stature. This is due to potential surgical problems and societal factors. In North America, as opposed to some European countries, it appears that society is more accepting of physically "different" individuals. Nevertheless, our environment is built for individuals of normal stature. Limb elongation for significant short stature cannot be considered merely cosmetic. Use of public facilities, including bus stops and washrooms, is extremely difficult for patients under 4 ft 6 in. Many patients cannot get onto toilets easily or use public sinks, drinking fountains, or cafeteria counters. These factors, however, must be weighed against the potential difficulties and complications of limb lengthening. It is difficult to lengthen both tibiae and femora without any complications and to be able to have them of equal length and appropriately aligned.

Finally, patient and family motivation and involvement must be considered. A strong commitment to this long and arduous process is necessary in order to achieve excellent functional and cosmetic results.

In 1997, the ideal short-statured candidate for limb lengthening is one for whom normal or nearly normal height can be achieved or for whom substantial lengthening will greatly enhance activities of daily living. The patient should have normal or nearly normal joints and adjacent musculature and must be psychologically prepared for and committed to a long, arduous process. Despite potential complications, the results to date have been encouraging, and continued judicious application of these techniques is warranted.

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Letter From the Editor

In *GROWTH, Genetics, & Hormones* (12 [3]:44), an article by Thalange et al, entitled "IGFBP-3 Generation: An Index of Growth Hormone Insensitivity," was published. Dr. Lifshitz and I each commented, and invited the authors to respond to our comments. They have done so, and their letter follows. You, the reader, may wish to refer to the original abstract and comments to better understand Dr. Clayton's reply.

Robert M. Blizzard, MD

Letter to the Editor:

There has been much recent interest in the clinical characterization, biochemical definition, and molecular analysis of the GH receptor (GHR) in congenital GH insensitivity (Laron syndrome). The classic biochemical criteria are not necessarily fulfilled by all patients who have the condition, and marked heterogeneity exists within the clinical phenotype and the degree of height retardation. This has instigated studies screening for GH receptor mutations in children classified as having idiopathic short stature (ISS). The finding of various heterozygous GH mutations within this population suggests that minor dysfunction within the GH receptor or its signaling pathways may be relevant to their short stature.

Our study, performed prior to the first reports of heterozygous GHR mutations in ISS, was intended to assess GH sensitivity (by IGF-1 and IGFBP-3 generation) in patients who were short (height SDS < -2) but had an arbitrarily defined high basal and/or peak GH level. Their bone ages were similar to chronologic age or slightly delayed, and 2 patients were in early puberty. A control group with GH deficiency was used for comparison.

Our data demonstrated the marked variability in response to acute administration of GH in both groups. This provides evidence for a wide spectrum of individual responsiveness to GH, regardless of the etiology of the short stature. The finding of GH receptor mutations in children with ISS, whose auxology is similar to those included in our proposed GH-insensitive group, adds further support to the concept of a range of GH sensitivity. We were not surprised, therefore, that we have not been able to define a precise biochemical tool to detect partial GH insensitivity. We suspect the definition of the latter from a biochemical perspective will prove as difficult as the definition of "partial GH insufficiency." Molecular analysis not only of the GH receptor, but also of other molecules involved in GH signaling is more likely to help us precisely locate the site(s) of GH insensitivity. Some children with unexplained short stature, in fact, may have a dysfunctional GH transduction pathway. An IGF-1/IGFBP-3 "generation test" can only provide useful supportive but not definitive evidence for such a diagnosis.

We appreciate the opportunity to respond to the editorial comments in the review of our article published in *GROWTH, Genetics & Hormones*.

Yours sincerely,
Dr. P. Clayton
Senior Lecturer in Child Health
Royal Manchester Children's Hospital
N.K.S. Thalange, et al

Abstracts From the Literature

A Receptor in Pituitary and Hypothalamus That Functions in Growth Hormone Release

The investigators cloned the endogenous receptor for the synthetic growth hormone-releasing peptides (GHRPs) and nonpeptidyl mimetics of GHRP (termed GH secretagogues [GHSs] by the authors). This receptor is present in the human, chimpanzee, swine, cattle, mouse, and rat pituitary, as well as in the arcuate, ventromedial, and infundibular regions of the primate (rhesus monkey) hypothalamus. There are 2 species of GHS receptors. The major GHS receptor (type Ia) is a 366 amino acid peptide with 7 transmembrane spanning domains coupled to G_{11} with apparently very short extracellular (42 amino acids) and intracellular (30 amino acids) domains. GHS receptor type Ib is a 289 amino acid protein with 5 transmembrane domains followed by a 24 amino acid intracellular domain. The GHS receptors have 30% identity with receptors for neurotensin and thyrotropin-releasing hormone (TRH). Radiolabeled MK-0677, a nonpeptidyl GHS, binds avidly to the GHS receptor and is displaced by unlabeled MK-0677, GHRP-2, and GHRP-6 with decreasing potency; GHRH, GnRH, TRH, ACTH, and galanin do not displace the radioligand.

Howard AD, et al. *Science* 1996;273:974-977.

Editor's comment: *Characterization of the endogenous receptor for the synthetic GHS validates further the presence of a third system involved in the regulation of pituitary GH secretion in addition to GHRH and SRIH. Utilizing the GHS receptor, it is now likely that its endogenous ligand will be identified. It is of interest that the GHS receptor is expressed not only in the pituitary but also in the hypothalamus. This finding confirms previous observations that the GHRPs act at both the pituitary and hypothalamic levels. The endogenous GHSs may be involved in the integration of GHRH and SRIH regulation of pituitary GH synthesis and/or secretion. (Besides the noteworthy scientific accomplishments reported in this paper, it was of interest to count the number of coauthors; 32 were listed.) This indeed was a team effort.*

Allen W. Root, MD

Intrauterine Growth Retardation and Postnatal Growth Failure Associated With Deletion of IGF-1

The authors report the case of a 15.8-year old boy referred for evaluation of short stature and growth delay in whom a diagnosis of GH insensitivity was suggested because of elevated basal and poststimulation GH levels, absent response to rhGH treatment, and low serum IGF-1 concentration. The patient was born at 37 weeks gestation with symmetric IUGR. His birth weight, length, and head circumference were 3.9, 5.4, and 4.9 SD below the mean, respectively. The placental weight was 1.3 SD below the mean. The patient had severe growth failure throughout infancy and childhood. At age 8 years, he underwent evaluation, which showed normal thyroid function tests, normal male karyotype, and elevated serum basal and peak GH levels (18 ng/mL and 94 ng/mL, respectively) after the administration of the clonidine. He received rhGH treatment for 1.7 years, starting at age 11 years, with no effect on his growth rate. IGF-1 levels performed at age 14 years were markedly below normal range (0.05 U/mL, normal for age = 0.48 to 2.8 U/mL). The patient was diagnosed with profound bilateral sensorineural deafness, moderately delayed motor development, hyperactivity, and short attention span. Additionally, some dysmorphic features were recognized, including micrognathia, bilateral ptosis, low hairline, and bilateral clinodactyly. The patient's parents were first cousins once removed. His father, mother, and 10-year-old sister had less severe growth impairment, with heights 1.8, 1.4 and 1.0 SD below the mean, respectively. Subsequent endocrine tests performed on the patient showed normal fasting blood glucose, thyrotropin, prolactin, and cortisol, and pubertal levels of DHEAS although pubic hair was at Tanner Stage 1. The gonadotropin response to GnRH was pubertal (patient was at Tanner Stage 2 for genitalia). The testosterone response to hCG was significant, and the GH peaks on the overnight GH secretion test were supernormal. A normal basal level of 2.2 ng/mL, but high poststimulation of 61 ng/mL levels of GH, were present. There was an absent response of IGF-1 in the generation test and normal IGF-2, IGFBP-3 and GHBP levels. Brain MRI studies were essentially normal, and electrophysiology studies of the CNS were also normal. Detailed DNA studies using PCR and reverse transcriptase PCR were able to identify homozygosity for the D12S346 polymorphism, consisting in the partial deletion of the IGF-1 gene at the level of the exons 4 and 5, and heterozygosity for such polymorphism in both parents and his sister. Both parents and his sister had low-normal levels of IGF-1 and normal IGF-2 and IGFBP-3.

Woods KA, et al. *N Engl J Med* 1996;335(18):1363-1367.

Editor's comment: *It has been known that GH has no direct impact on intrauterine growth. Indirect evidence suggests that insulin, IGF-1, and IGF-2 are the possible mediators of intrauterine growth. This report yields evidence for the pivotal role of IGF-1 in the process of intrauterine and postnatal growth. Unfortunately, this patient was not treated with IGF-1 to ascertain the growth response to this hormone. The coexistence of postnatal growth failure with a history of IUGR should prompt us to think of problems in the expression or action of IGF-1. IUGR patients who do not exhibit catch-up growth need to be assessed, as was this patient. Another interesting finding of this case is the description of less severe growth retardation in the parents and the sister of the proband, all of whom had normal levels of IGF-1, IGF-2, and IGFBP-3. We must ask: "How many individuals do we see with moderate growth failure and without definitive GH alterations who might be cases of heterozygous deletions of portions of the IGF-1 gene?"*

Fima Lifshitz, MD

Newborn Screening Fact Sheets

The Committee on Genetics of the American Academy of Pediatrics has developed and published fact sheets regarding newborn screening that are very useful resources for physicians dealing with children who have metabolic disorders. These guidelines were designed to help physicians understand and interpret the various tests employed for newborn screening. They take into consideration the variations in screening procedures in different states, and give information concerning early detection, treatment, and follow-up of infants with metabolic disorders. For the purpose of counseling and referral, the information also covers the identification of asymptomatic "carrier couples."

The availability of newborn screening is discussed, and professional and public educational materials are suggested. References for additional reading, notes on early hospital discharge, and costs in each state also are provided.

There is detailed information about biotinidase deficiency; maple syrup urine disease; congenital adrenal hyperplasia; congenital hypothyroidism; cystic fibrosis; galactosemia; homocystinuria; phenylketonuria; sickle cell disease; toxoplasmosis; and tyrosinemia. For each condition, the following are reviewed: State Newborn Screening Availability; Brief Clinical Description; Genetics (including chromosomal map location, incidence, inheritance, racial and ethnic variability,

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GROWTH
Genetics & Hormones

Dear Colleagues:

The field of genetics is where the action is today. The members of the Editorial Board have been aware for some time that the fields of genetics, pediatric endocrinology, nutrition, and growth are intimately intertwined. This knowledge prompted establishment of *GROWTH, Genetics, & Hormones* to stimulate and facilitate intellectual exchange of important knowledge among these disciplines. Drs. William Horton and Judith Hall have been key in representing members of the genetic subspecialty on our Editorial Board. The glossary that you are holding in your hands, which is an updated revision of the one first published in *GGH* Vol 9, No. 1, 1993, results from their efforts to simplify and interpret terms that recently have appeared in the vocabulary of geneticists. This they have done to permit us to more readily understand that which we read. We thank them for their effort and contribution.

We also thank Genentech, Inc. for the additional funds placed in our educational grant so this glossary can be brought to you.

Please note that this glossary is physically separate from the remainder of the publication. This is by design to permit you to readily access the information in the glossary when you need it in interpreting the articles that you will read in the future, both in *GROWTH, Genetics, & Hormones* and elsewhere.

We hope this endeavor constructively assists you in quickly understanding more fully the important and pertinent articles that will be appearing in future issues of *GROWTH, Genetics, & Hormones*.

Respectfully,
For the Editorial Board

Robert M. Blizzard, MD
Editor

GROWTH
Genetics & Hormones

GENETICS GLOSSARY

acceptor splice site The boundary between the 3' end of an intron and the 5' end of the adjacent exon.

allogeneic The allelic variation seen among members of the same species.

anticipation Phenomenon in which the severity of a genetic condition appears to become more severe and/or arise at an earlier age with subsequent generations (seen in many trinucleotide repeat permutations).

ascertainment The selection of individuals for inclusion in a genetic study (severity, age of onset, certain features of the trait).

apoptosis Programmed cell death; a physiologic process conserved to remove unwanted cells.

association In a specific population, the occurrence together of 2 or more different phenotypes more often than expected by chance.

ATP Abbreviation for adenosine triphosphate. The energy-yielding molecule in cells that is used to drive chemical reactions.

autophagy Digestion of the cell's own organelles.

autosome Any chromosome other than the X or Y. Humans have 22 pairs of autosomal chromosomes.

autosomal disease A disease encoded by a gene on one of the 22 pairs of autosomes.

autosomal dominant A trait that is expressed in an individual who is heterozygous for a particular gene when the mutant allele is on one of the autosomes.

autosomal modifier gene A gene that modifies the action of the autosomes.

autosomal recessive A trait that is expressed in an individual who is homozygous for a particular gene.

BAC See bacterial artificial chromosome.

backcross A genetic crossing of a heterozygous organism and one of its homozygous parents.

bacterial artificial chromosome (BAC) Artificial chromosome vector derived from bacteria used for cloning relatively large DNA fragments.

balanced translocation A rearrangement translocation with no apparent loss or gain of chromosomal material, resulting in a clinically normal but genetically "abnormal" person.

banding The differential staining of a chromosome by a variety of techniques that results in a specific pattern of positively and negatively stained bands for each chromosomal pair.

base analogue A substance that can mimic the chemical behavior of 1 of the 4 DNA bases.

base pair substitution The replacement of 1 base pair by another.

Barr body The sex chromatin mass located adjacent to the nuclear membrane in interphase nuclei, which corresponds to an inactivated X chromosome. One Barr body is seen in the cells of 46,XX and 47,XXY individuals, and none in the cells of 45,X and 46,XY individuals.

Bayesian analysis Mathematical method for calculating probability of the carrier state in mendelian disorders by combining several independent likelihoods.

bioinformatics The discipline of using computers to address information problems in the life sciences; it involves the creation of electronic data bases on genomes, protein sequences, etc.

bivalent A pair of homologous chromosomes in association as seen at metaphase of the first meiotic division.

CAG/CTG repeats Abbreviation for cytosine-adenine-guanine triplet nucleotide and cytosine-thymine-guanine triplet nucleotide repeats; they are associated with unstable mutations.

candidate gene A gene known to be located in the region of interest whose product has biochemical or other properties suggesting that it may prove to be the disease gene being sought.

candidate gene approach Strategy to identify disease-associated genes based on finding candidate genes in a chromosome region in which a disorder is mapped.

cap A modified nucleotide added to the 5' end of a growing mRNA chain, apparently required for normal processing, stability, and the translation of mRNA.

cap site The site of initiation of transcription.

CAT assay Reporter gene assay used to measure activity of a promoter under different conditions, such as to define elements of a promoter or to study signals that activate an intact enhancer/promoter. CAT is the abbreviation for the enzyme, chloramphenicol acetyl transferase, the activity of which is measured in the assay.

cell line A cultured cell type that can be reproduced indefinitely, ie, immortalized.

CG island Unmethylated cytosine-guanine sequences that are often found near the 5' ends of some genes.

chain termination mutation A mutation that generates a stop codon, thus preventing further synthesis of the polypeptide chain.

chromosome aberration An abnormality of chromosome number or structure.

clinical genetics That part of medical genetics concerned with health and illness in individuals and families.

clinical heterogeneity Refers here to the production of clinically different phenotypes from mutation in the same gene.

codominance The expression of both alleles in a heterozygous individual, eg, presence of both hemoglobin A and S on electrophoresis in an individual heterozygote for sickle-cell disease.

complementary DNA (cDNA) DNA synthesized from an mRNA template, using reverse transcriptase.

complementation analysis A genetic test for determining whether 2 mutations producing a similar phenotype are allelic.

concordance Presence of the same trait in both members of a pair of twins (or set of individuals).

confined placental mosaicism Mosaicism that is seen only in the placenta but not in the fetus.

congenic mouse strain A strain that differs from another in the region containing 1 genetic locus.

consultand Individual seeking, or referred for, genetic counseling.

contig A series of contiguous, overlapping, cloned DNA fragments.

copy number The number of copies of a transgene integrated into a host genome; used to describe transgenic animals.

crossing over Reciprocal breaking and rejoining of homologous chromosomes in meiotic prophase I that results in exchange of chromosomal segments.

deletion Loss of part or a whole chromosome or loss of DNA nucleotide bases.

dicentric Refers to an aberrant chromosome that contains 2 centromeres.

diploid The number of chromosomes in most somatic cells, which is double the number found in the gametes (the haploid number). In humans, the diploid chromosome number is 46.

discordant A twin pair (or set of individuals) in which one member exhibits a certain trait and the other does not.

dizygotic The product of fertilization of 2 separate eggs by 2 separate sperm; nonidentical twin pair.

DNA construct A DNA sequence that has been modified to yield a recombinant DNA molecule.

DNA ligase Enzyme that catalyzes religation (reconnection) of 2 fragments of DNA.

DNA rearrangements Recombination of DNA segments, eg, in cells of the immune system, the variable (V), diversity (D), and joining (J) regions somatically rearrange to generate functional antibody genes.

dominant (trait) Those conditions that are expressed in heterozygotes, ie, individuals with 1 copy of the mutant gene and 1 copy of the normal allele; refers to phenotype.

double heterozygote An individual with 1 mutant allele at each of 2 different loci.

donor site Guanine-thymidine sequence that defines the splice site at the 5' end of an intron.

duplication The presence of an extra copy of chromosome material. At the gene level, this refers to the presence of more than 1 copy of a structured gene, usually having arisen through unequal crossing over. At the chromosomal level, this refers to an unbalanced state in which there may be a triple dose of a portion of an autosome, usually occurring as the result of unequal segregation of a translocation in meiosis (trisomy).

ecogenetic disorder A disorder resulting from the interaction of a common environmental factor with a specific genetic predisposition, eg, cigarette smoking causing emphysema in alpha$_1$-antitrypsin deficiency.

electroporation Application of a short, high-voltage electric pulse to cells in the presence of DNA to permit DNA to enter the cells.

embryo biopsy Potential method for preimplantation diagnosis of genetic disorders used in conjunction with in vitro fertilization in which cells are removed and analyzed at a very early stage in embryonic development.

embryonic stem cells Cells derived from early embryos that can replicate indefinitely and differentiate into many cell types. Stem cells serve as a continuous source of new cells; they may become incorporated into many tissues to produce chimeric animals when introduced into early embryos, ie, blastocysts.

empiric risk Risk of recurrence for multifactorial or polygenic disorders based on family studies (observed risk).

endonuclease Enzyme that cleaves bonds between nucleotides of single- or double-stranded DNA or of RNA at specific sequences of nucleotides.

env gene Encodes capsule or envelope protein of a retrovirus.

epigenetic A factor that changes the phenotype without changing the genotype.

episome A plasmid that can exist either independently in the cytoplasm or as an integrated part of the genome of its bacterial host.

ES cells See embryonic stem cells.

EST See expressed sequence tag.

exonuclease An enzyme that cleaves nucleotide chains at their terminal bonds only.

expressivity The degree to which a heritable trait is expressed in an individual. "Variable expressivity" refers to the variation in phenotype and in severity produced by the same gene in different individuals.

expressed sequence tag A short fragment of an expressed sequence, cDNA, which serves as a landmark for gene mapping.

expression The observable effects of an active gene.

F_1 hybrids The first generation of animals generated from 2 different inbred strains. These animals are genetically identical to one another but different from either inbred parent.

F_2 hybrids The progeny produced from matings between F_1 animals. These animals are different from one another and will contain different mixtures of the genetic variations that were present in the original inbred progenitors.

flanking sequence A region of a gene preceding or following the transcribed region.

footprinting (DNA footprinting) Assay used to study DNA-binding proteins.

founder Refers to animals generated from genetically altered eggs or embryos, ie, eggs microinjected with a transgene.

founder effect The high frequency of a mutant gene in a rapidly expanding population founded by a small ancestral group when 1 or more of the founders were, by chance, carriers of the mutant gene.

gel-shift assay An assay used to detect specific protein binding to DNA. Such binding creates complexes that migrate more slowly during gel electrophoresis than free DNA. Also known as mobility-shift assay.

gene family A group of genes having similar DNA sequence evolved from a single ancestral gene. These genes may or may not be located in the same region of a chromosome.

genetic code The base triplets that specify the 20 different amino acids.

gene flow Gradual diffusion of genes from one population to another, as a result of migration and intermarriages.

gene therapy A strategy in which therapeutic genes are introduced into a person's cells to correct a disease or genetic flaw.

genetic drift Random fluctuations in gene frequencies, most evident in small populations.

genetic heterogeneity Different mutations causing a similar phenotype; allelic heterogeneity refers to different mutations at the same locus, whereas locus heterogeneity refers to mutations at different loci.

genetic lethal A genetic disease that prevents fertility.

genetic mapping Determination of the relative positions of genes on a DNA molecule (chromosome or plasmid); distances are measured in linkage units, ie, centimorgans (cM), between them.

genetic marker A polymorphic genetic property that can be used to distinguish the parental origin of alleles.

haplotype A set of closely linked genes that tends to be inherited together as a unit, as occurs with the A, B, and C loci of the human leukocyte antigen (HLA) gene complex.

HA Abbreviation for heteroduplex analysis.

heteroduplex Refers to a region of a double-stranded DNA molecule with noncomplementary strands that originated from different duplex DNA molecules.

heteromorphism A normal morphologic or staining variant of a chromosome.

heteroplasmy The existence of more than 1 mitochondrial type in the cells of an individual, ie, the presence of both normal and mutant mt DNA in a single individual.

heteroploid An individual with an abnormal number of chromosomes (as compared to euploid, which is the normal number of chromosomes).

heterotetramer A molecule consisting of 4 subunits, at least 1 of which differs from the others.

homoplasmy The presence of a single population of mt DNA in the cells of a single individual. This is normal.

homotetramer A molecule consisting of 4 identical subunits.

hybrid cell A cell formed by fusion of 2 cells of different origin in which the 2 nuclei have merged into 1. Can be cloned to produce hybrid cell lines.

inbred mouse strain A strain of mice that has been maintained by successive brother to sister matings over many generations, eg, BALB/c and C57BL/6 mice strains.

inducer A molecule that induces the expression of a gene.

initiation factor A protein that associates with the small subunit of a ribosome when protein synthesis begins.

insertional mutagenesis The production of a mutation by insertion of 1 or more copies of a transgene into a host genome.

in situ Refers to carrying out experiments or tests with intact tissues.

intergenic DNA The untranscribed DNA of unknown function that makes up a large proportion of the total DNA.

inversion A structural rearrangement of a chromosome in which 2 breaks occur, followed by the reinsertion of the chromosome segment but in reversed order. It may be either paracentric, ie, it does not include the centromere, or pericentric, ie, it does include the centromere.

in vitro Refers to a biologic or biochemical phenomenon that occurs outside of a living organism.

in vivo Refers to a biologic or biochemical phenomenon that occurs within a living organism.

isochromosome A structural chromosome rearrangement caused by the division of a chromosome along an axis perpendicular to the usual axis of division; results in chromosomes with either 2 short arms or 2 long arms.

isodisomy The presence of 2 identical homologues of a transmitted chromosome from only 1 of the parents.

junk DNA DNA with no apparent function.

kinetochore A structure at the centromere to which the spindle fibers are attached.

lagging strand of DNA The new strand of a DNA replicating in the 3' to 5' direction. It is synthesized in short fragments in the 5' to 3' direction that are subsequently joined together.

lethal factor An abnormality of the genome that leads to death in utero, eg, numerous chromosomal anomalies.

ligand A molecule that can bind to a receptor and thereby induce a signal in the cell, eg, a hormone.

linker DNA A synthetic DNA that carries the recognition site for a restriction enzyme and that can bind 2 DNA fragments. Also, the stretch of DNA between 2 nucleosomes.

linkage map A chromosome map showing the relative positions of genetic markers of a given species, as determined by linkage analysis; not the same as a physical, or gene, map, which uses linkage analysis, cytogenetic examination, and physical techniques to generate the map.

linkage phase The arrangement of alleles of linked loci on chromosomes.

loss of heterozygosity Describes a locus (or loci) at which a deletion or other process has converted the locus from heterozygosity to homozygosity or hemizygosity. Phenomenon can lead to cancers by loss of tumor suppressor genes.

lyonization A term used for the phenomenon of X inactivation, which was first proposed by the geneticist Mary Lyon.

Maxam-Gilbert method A method for determining the exact nucleotide sequence via a chemical degradation process.

mendelian inheritance A trait obeying Mendel's first law of independent segregation of the alleles at the same locus conveyed by each parent.

minimal promoter The minimal elements of a promoter, including the TATA box and transcription initiation site, which is inactive unless regulatory elements that enhance promoter activity are placed upstream; used to test candidate sequences for enhancer activity.

mismatch The presence in 1 chain of double-stranded DNA of a base that is not complementary to the corresponding base in the other chain. Also known as mispairing.

mitochondria A small, intracellular, spherical to rod-shaped cytoplasmic organelle, enclosed by 2 membranous spaces; the inner membrane is folded, forming a series of projections called cristae. Mitochondria are the principal sites of ATP synthesis; they contain enzymes of the tricarboxylic acid cycle and enzymes for fatty acid oxidation, oxidative phosphorylation, and many other biochemical pathways. They contain their own nucleic acids and ribosomes, replicate independently, and code for the synthesis of some of their own proteins.

molecular hybridization The ability of a single-stranded DNA or RNA to anneal to its complementary single strand by Watson-Crick base pairing.

mobile elements DNA sequences that are capable of inserting themselves into other locations in the genome.

mobility-shift assay An assay used to detect specific protein binding to DNA. Such binding creates complexes that migrate more slowly during gel electrophoresis than free DNA. Also known as gel-shift assay.

modifier gene A gene that alters the expression of a gene at another locus.

molecular genetics The study of the structure and function of genes at the molecular level.

monoclonal A group of cells that consist of a single clone, ie, all cells are derived from the same single ancestral cell.

monogenic Describing a single gene or mendelian trait.

morphogen A protein present in embryonic tissues in a concentration gradient that induces a developmental process.

mosaic An individual or tissue with at least 2 cell lines differing in genotype or karyotype, derived from a single zygote.

monosomy An aneuploid condition in which a specific chromosome is present in only single copy, giving the individual a total of 45 chromosomes.

monozygotic Refers to twins derived from a single fertilized egg.

multipoint mapping A type of genetic mapping in which the recombination frequencies among 3 or more loci are estimated simultaneously.

murine Relating to mice or rats.

mutagen A substance that causes a mutation.

neurofibromin The protein product of the neurofibromatosis type 1 gene.

new mutation An alteration in DNA sequence that appears for the first time in a family as the result of a mutation in 1 of the parent's germ cell.

nondisjunction The failure of homologous chromosomes (in mitosis or meiosis I) or sister chromatids (in meiosis II) to separate properly into different progeny cells.

nonpenetrance Lack of clinical expression of the mutant phenotype in an individual with the appropriate genotype.

nuclear family A pair of biologic parents and their children.

nude mice Immunologically deficient mice used to permit growth of tumor cells from mouse and other species, such as human.

null mutation An allele that results in either the absence of the gene product or the absence of any function at the phenotypic level.

obligate heterozygote An individual who is clinically unaffected but, on the basis of pedigree analysis, must carry a particular mutant allele.

oligogenic diseases Diseases or traits that result from the effects of relatively few genes, some of which have rather large effects.

oligoprobe A short DNA probe whose hybridization is sensitive to a single base mismatch.

oncogenes Normal genes of vertebrates that are involved in control of cell growth and have been preserved throughout evolution. When mutated, overexpressed, or amplified in somatic cells, oncogenes may cause neoplastic transformation.

organelles Membrane-bound intracellular, cytoplasmic structures having specialized functions, eg, mitochondria, plastids, Golgi apparatus, lysosomes.

origin of replication (ORI) The site where DNA replication starts.

outbred mouse strains Strains of mice propagated by nonstandardized matings. These mice retain substantial genetic variability.

PAC The artificial chromosome vector derived from the temperate bacteriophage, P1, used for cloning 100- to 200-kb DNA fragments.

palindrome In molecular biology, a nucleotide sequence in which the 5' to 3' sequence of 1 strand of a segment of DNA is the same as that of its complementary strand. The sites of many restriction enzymes are palindromes.

PEP Abbreviation for primer extension preamplification.

peptide fingerprint The chromatographic pattern of peptides obtained after partial hydrolysis of a protein or peptide. The technique also may be applied to DNA and RNA.

peroxisomal enzymes Enzymes localized to the peroxisomes. These enzymes are initially synthesized by the free polyribosomes and then enter the cytoplasm and eventually are localized to the peroxisomes. There are at least 40 enzymes. Some are involved in the production and decomposition of hydrogen peroxide and some are concerned with lipid and amino acid metabolism.

peroxisome A subcellular organelle surrounded by a single membrane containing at least 40 enzymes involved in energy production.

physical mapping The determination of the linear positions of genes on a DNA molecule; distances are measured in physical units, ie, base pairs, kilobases, and megabases.

phytohemagglutinin Lectin isolated from the red bean used to agglutinate red blood cells and stimulate lymphocytes to divide; used in preparation of peripheral blood karyotypes.

platelet-derived growth factor (PDGF) A protein, produced by platelets and other cells, that strongly stimulates cell growth and division and is involved in normal wound healing. The gene for PDGF is identical to the proto-oncogene *sis*.

pulsed field electrophoresis An electrophoretic technique that allows the separation of relatively long (>5,000 kb) sequences of DNA.

point mutation A mutation in a single nucleotide.

polyadenylation The addition of approximately 200 adenosine residues at the 3' end of messenger RNAs, apparently involved in their transport of the nucleus and stability.

polymerases Enzymes that catalyze the combining of nucleotides to form RNA or DNA (genetic transcription and DNA replication).

polysomes (polyribosomes) Structures composed of multiple ribosomes attached to mRNA in the process of translation.

pronucleus Either of the 2 haploid gamete nuclei just prior to their fusion in the fertilized ovum. Transgenic lines are often generated by microinjection of the transgene into the pronuclear region of these haploid gametes.

proofreading The correction of errors in the nucleotide sequence that can occur during replication, transcription, or translation.

protein suicide mechanism In dominant disorders, 1 mutant subunit leads to the loss of function of an entire multimeric protein, eg, collagen.

proto-oncogenes Normal genes that are found in normal eukaryotic cells concerned with various aspects of cell division. If amplified, mutated, rearranged, or picked up by a retrovirus, they may give rise to oncogenes that can cause cancer.

pseudoautosomal region The distal tip of the Y chromosome short arm, which undergoes crossover with the distal tip of the X chromosome short arm during meiosis in the male.

quasidominance The pattern of inheritance produced by the mating of an affected homozygote with an individual heterozygous for the same recessive trait so that homozygous affected members appear in 2 or more successive generations.

Q-banding The pattern of bright and dim fluorescent crossbands seen on chromosomes under ultraviolet light after quinacrine mustard staining.

R-banding A chromosome banding technique in which chromosomes are heated in a phosphate buffer; produces dark and light bands in patterns that are the reverse of those produced by G-banding.

receptor A transmembrane or intracellular protein involved in transmission of a cell signal.

recombinant chromosome A chromosome in an offspring that has a genotype not found in either parent, due to crossing over in meiosis.

recombination fraction In linkage analysis, the fraction of meiotic events that show a recombination between 2 loci.

regulatory gene A gene coding for a protein that regulates other genes.

replication The identical duplication of DNA.

replication fork The unwound region of the DNA double helix in which replication takes place.

replication segregation Refers to changes in the proportions of mitochondrial DNA alleles as the mitochondria reproduce.

reporter gene A gene used to analyze another gene.

restriction digest The process in which DNA is exposed to restriction enzymes (restriction endonuclease), causing it to be cleaved into fragments of DNA called restriction fragments.

restriction map A map of a DNA sequence with restriction enzyme recognition sites serving as landmarks.

restriction site A short sequence in DNA that can be recognized and cut by a specific restriction endonuclease.

reverse genetics The application of human gene mapping to clone the gene responsible for a particular disease when no information about the biochemical basis of the disease is available.

ribosomes Cytoplasmic organelles composed of ribosomal RNA and protein, on which polypeptide synthesis from messenger RNA occurs.

ring chromosome A structurally abnormal chromosome in which the end of each chromosome arm has been deleted and the broken arms have reunited to form a ring.

RT- PCR Abbreviation for reverse transcriptase polymerase chain reaction.

Sanger method The enzymatic method for determining the exact nucleotide sequence of a cloned fragment of DNA.

satellite DNA A portion of the DNA that differs enough in base composition so that it forms a distinct band on cesium chloride gradient centrifugation; usually contains highly repetitive DNA sequences.

scaffold The nuclear structure observed when histones are experimentally removed from chromosomes. Thought to represent a structural component of the nucleus and of chromosome.

segregation The separation of allelic genes at meiosis. Because allelic genes occupy the same locus on homologous chromosomes, they pass to different gametes.

sequence-tagged site (STS) A short fragment of DNA whose exact sequence is found nowhere else in the genome; typically about 200 to 300 bp. Polymerase chain reaction can be used to amplify the known sequences, which can serve as physical landmarks for mapping.

sibship The group comprising all the siblings (brothers and sisters) in a family.

silent gene A mutant gene that has no detectable phenotypic effect.

silencer The *cis* regulatory element that reduces transcription of a gene.

site-directed mutagenesis The process of creating mutations at specific locations, in contrast to naturally occuring random mutations.

skewed X-inactivation A nonrandom pattern of inactivation of 1 of the X chromosomes in a female that can arise through a variety of mechanisms. When this occurs, the active X chromosome may bear the mutant allele and the female will show signs and symptoms of the disease. The female is called a manifesting heterozygote or a carrier.

somatic cell gene therapy The insertion of new DNA material into a particular tissue of an affected individual in such a way that the inserted DNA does not enter the germline.

SSCP Abbreviation for single-strand conformation polymorphism.

SSP Abbreviation for sequence-specific primer. These are used in PCR reactions.

STR Abbreviation for short tandem repeat. These often serve as polymorphic markers.

STS See sequence-tagged site.

STRP Abbreviation for short tandem repeat polymorphism.

syngeneic Refers to genetically identical members of the same species.

transcript map A genetic map in which expressed sequences, eg, mRNAs, mRNA transcripts, corresponding to genes are mapped; a functional blueprint of the genome.

transforming retrovirus A retrovirus carrying an additional DNA sequence (often an oncogene) that confers the ability to transform infected cells to malignant phenotype.

transgene A foreign gene; typically, a gene produced by recombinant DNA techniques.

transposable element A DNA sequence that can move from one chromosomal location to another.

transversion A mutation in which purine is substituted for pyrimidine or vice versa.

triplet A sequence of 3 nucleotides comprising a codon of a nucleic acid and representing the code for an amino acid (triplet code, codon).

tumor-suppressor gene A gene thought to suppress formation of tumors; loss of suppression leads to malignant transformation. P53 is an example of a tumor-suppressor gene.

unequal crossing over Crossing over between similar DNA sequences that are misaligned, resulting in sequences with deletion or duplication of DNA segments. A cause of a number of genetic variants, eg, α-thalassemia and Lepore hemoglobin.

variable expressivity Refers to the variable severity of a genetic trait. Individuals with the same mutant gene with pleiotropic effects frequently show variable expressivity due to either environmental effects or effects of other genes modifying the expression of the mutant gene.

wild type The term used to indicate the normal allele (often symbolized as +) or the normal phenotype.

X-autosome translocation The reciprocal translocation between the X chromosome and 1 of the autosomes.

X-linked dominant A trait that is manifested in the heterozygous female as well as in the male who has the mutant allele on 1 of the X chromosomes.

X-linked recessive A disorder manifested exclusively in a male who is a heterozygote or a homozygous female when the abnormal gene is carried on the X chromosome. A female is usually a carrier if she is heterozygous and transmits the disease to the son.

zinc finger proteins Transcription-activator proteins containing finger-like structures containing zinc atoms.

zoo blot A Southern blot containing conserved DNA sequences from related genes of different species. It is taken as evidence that the sequences are coding sequences from a specific gene.

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molecular pathology, potential for symptomatic diagnosis, genotype-phenotype correlation, and genetic counseling); Severity and Variability Without Screening (including mortality, developmental disabilities, and physical findings); Clinical Outcome With Screening and Treatment (including mortality, clinical disability, variability, and possible interventions); Screening Test Characteristics and Confirmation (including type of test, timing, stability of specimen, confirmation, accuracy of screening, and ongoing studies); Special Concerns and Issues; and Professional and Public Education.

Information on additional newborn screening tests that are available in some university-based laboratories and commercial laboratories also is provided. These include adenosine deaminase deficiency; arginase deficiency; urea cycle defects; Duchenne muscular dystrophy; glucose-6-phosphate dehydrogenase deficiency; pyroglutamic aciduria; medium-chain acetyl-CoA dehydrogenase deficiency; and other organic acidemias.

American Academy of Pediatrics, Committee on Genetics. *Pediatr* 1996;98(3):473-500.

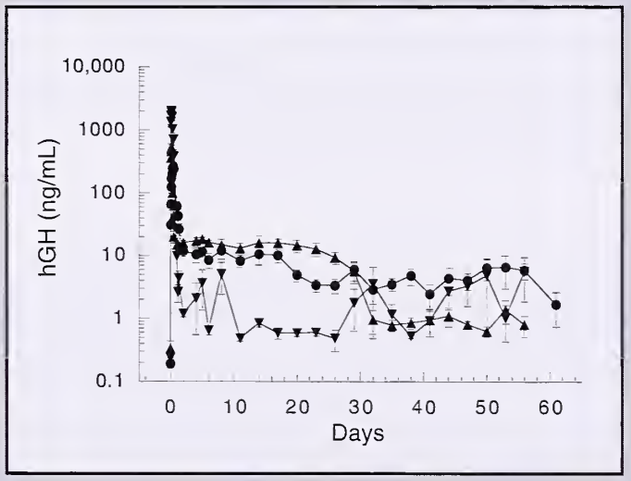
Editor's comment: *This is a useful resource for pediatricians who suddenly find they need information about newborn screening. Information regarding many different metabolic disorders is contained in this one article. Each section provides current information on screening variability among different states, appropriate therapies, as well as the recent advances in genetics regarding metabolic disorders. The morbidity and mortality of metabolic diseases can be improved significantly with early detection and treatment. The field of metabolic diseases is changing rapidly because of molecular genetic techniques and new types of therapy. These guidelines will help physicians help their patients. Obtain your copy soon.*

Judith G. Hall, MD

A Month-Long Effect From a Single Injection of Microencapsulated Human Growth Hormone

The investigators have prepared a sustained-release form of rhGH using zinc and incorporating it into biodegradable polymers of DL-lactic co-glycolic acid (PLGA), producing 50-µm diameter microspheres. The monomeric form of rhGH is released from these microspheres. One subcutaneous (sc) injection did not cause an inflammatory reaction or fibrosis at the site of injection. When this microencapsulated form of rhGH (24 mg) was injected sc into juvenile rhesus monkeys, the peak serum rhGH concentration (260 ng/mL) was achieved within 12 hours after injection. Levels of rhGH declined and were maintained at 10 ng/mL through day 20 and thereafter at 4 to 5 ng/mL through day 60 after administration (this is a calculated rate of release of rhGH from the microspheres 0.4 mg/d) (see Figure 1). Insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) values increased 2- to 3-fold within 3 days and were maintained for 30 days. These data were similar to those recorded in another group of animals receiving microsphere-equivalent amounts of rhGH by osmotic pump. Daily injections of rhGH (0.86 mg/d) resulted in lower levels of IGF-1 and IGFBP-3. One of 4 animals developed a low titer of anti-rhGH antibodies.

Figure 1



Recombinant hGH serum concentration levels in rhesus monkeys. Values are means ± SEM. Treatment groups were 160 mg microspheres (24 mg rhGH) (●), 24 mg rhGH in solution (▼), or 3.4 mg rhGH in solution followed by surgical implantation of an osmotic pump containing 20.8 mg rhGH in solution (for a total dose of 24 mg) (▲).

From Johnson OL, Cleland JL, Lee HJ, et al. A month-long effect from a single injection of microencapsulated human growth hormone. *Nat Med* 1996;2(7):797.

Johnson OFL, et al. *Nat Med* 1996;2(7):795-799.

Editor's comment: *The availability of a clinically useful preparation of hGH that can be administered once a month or less will be of great benefit in the management of patients with GH deficiency and analogous to the utility of depot forms of GnRH agonist in central precocious puberty. If further studies demonstrate the safety and effectiveness of this preparation of rhGH, it may have more utility than oral forms of GH secretagogues, which need to be taken at least once daily and which will be ineffective in patients with primary pituitary dysfunction.*

Allen W. Root, MD

Over Expression of an Osteogenic Morphogen in Fibrodysplasia Ossificans Progressiva

Fibrodysplasia ossificans progressiva was first described in 1692. It is a rare autosomal dominant inherited disorder of the connective tissue with a high rate of new spontaneous mutations. Most cases represent new mutations and are sporadic. The disorder is characterized by short great toes, broad femoral necks, short metacarpals, and, with age, progressive formation of ectopic bone in the soft tissue and muscles. Ectopic bone formation is usually provoked by trauma and can occur at any age but is most often first seen between birth and 10 years. A significant number of patients with fibrodysplasia ossificans progressiva have heterotopic ossification at injection sites following DPT vaccinations.

The disorder is usually progressive, with a characteristic pattern of involvement. There is a predilection for developing ossification in the paraspinal and scalp muscles, jaw muscles, and muscles of the arms and legs; however, the facial muscles, muscles of the tongue, diaphragm, visceral smooth muscles, and abdominal muscles are usually spared. The disorder eventually leads to ankylosis (fixation of joints); severe scoliosis (from ossification of paravertebral muscles); starvation (from involvement of the jaw muscles); and premature death due to complications of ankylosis.

The authors speculate that overexpression of bone morphogenetic protein 4 in lymphocytes of patients with fibrodysplasia ossificans progressiva is the causative factor for heterotopic ossification in these individuals. They also suggest a mechanism to explain the pathophysiology of heterotopic bone formation in these individuals.

Bone morphogenetic proteins are potent osteogenic agents that belong to the transforming growth factor β (TGF-β) family, a superfamily of peptides that is responsible for endochondral osteogenesis and fracture healing. The gene for bone morphogenetic protein 4 has been mapped to chromosome 14q22-23.

The authors examined the expressions of bone morphogenetic proteins 1 to 7 as well as their mRNAs in the lymphoblastic cells of individuals with fibrodysplasia ossificans progressiva and in normal healthy individuals. They found overexpression of bone morphogenetic protein 4 and its mRNA in lymphoblastic cell lines from 26 of 32 individuals with fibrodysplasia ossificans progressiva and increased expression in 1 of 12 normal individuals. The authors speculate that following an injury, those lymphocytes having increased bone morphogenetic protein 4 migrate to the site of trauma to aggregate in large numbers. They postulate that these lymphocytes then bind to the type IV collagen present in the basement membrane of endothelial cells and muscle cells. This binding leads to a high local concentration of the bone morphogenetic protein 4, triggering preosseous fibroproliferative lesions.

Bone morphogenetic protein 4 is markedly increased during embryonic life as well as later on in development when healing of fractures occurs. This suggests a common molecular basis for prenatal and postnatal osteogenesis. Fibrodysplasia ossificans progressiva is the only known genetic disorder of osteogenesis that is associated with overexpression of a bone morphogenetic protein. An error could be in the regulatory regions of the *BMP-4* itself or in some other gene whose product controls *BMP-4* production. The commentary by J.M. Connor in the same issue of the journal is helpful but speculative in predicting care of patients with fibrodysplasia ossificans progressiva. Readers who wish more information should seek out and read this commentary.

Connor JM. *N Engl J Med* 1996;335(8):591-593.
Roush W. *Science* 1996; 273(30):1170.
Shafritz AB, et al. *N Engl J Med* 1996;335(8):555-561.

***Editor's comment**: Fibrodysplasia ossificans progressiva provides a unique opportunity to study the role of morphogenetic proteins in endochondral bone formation. Whether the overexpression is due to a defect in the gene itself or in the receptors needs to be determined. Once this is known perhaps these proteins can be modified therapeutically to turn off the expression of bone morphogenetic protein and thereby decrease ectopic bone formation.*

Understanding the biology of these proteins will have major clinical and therapeutic implications. It will help define the etiology and mechanism of ectopic bone formation in other diseases, such as soft-tissue ossification (myositis ossificans), which may occasionally occur as a complication of major or repeated minor muscle trauma, hip replacement, major burns, immobilization after paraplegia, or prolonged comas in otherwise healthy subjects.

It also seems possible that once their biology is known, these proteins might be useful in tissue engineering and may even possibly replace bone grafts in the future.

Judith G. Hall, MD

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Growth Hormone Therapy in Silver-Russell Syndrome: 5 Years Experience of the Australian and New Zealand Growth Database (OZGROW)

This report details the experience of 33 patients (22 males, 11 females) who were diagnosed with the Silver-Russell syndrome through the OZGROW data base and subsequently treated with exogenous growth hormone (GH) for 3 to 5 years. The inclusion criteria for Silver-Russell syndrome were: (1) short stature 2 standard deviations (SD) or more below the mean; (2) birth weight 2 SD or more below the mean for gestational age; and (3) lack of other recognized syndrome or etiology causing short stature. Of the 33 patients, 23 had at least one GH stimulation test, and 2 of the 23 had GH deficiency defined as a peak of <20 mIU/L in 2 different tests. Subjects were eligible for GH therapy if their height was less than the 1st percentile and their growth velocity was less than the 25th percentile for bone age and sex (the guidelines for GH therapy in Australia and New Zealand). The children received subcutaneous GH 6 or 7 days per week at a starting dose of 14 IU/m^2/wk (approximately 4.6 mg/wk). Growth was assessed every 3 months and bone age was assessed yearly. The dose was subsequently increased at 6-month intervals if the growth response was considered inadequate.

The median age at commencement of treatment was 6.7 years, and the median height SD score (SDS) for chronologic age was -3.2. The median birth weight and the median birth length for gestational age were -3.2 and -4.0 SD, respectively. Bone age was delayed in girls by 0.8 years and in boys by 2.2 years. Height SDS for chronologic age, growth velocity (cm/y), and SDS growth velocity for chronologic age increased during therapy. The median change in height SDS for chonologic age after 3 years of therapy (N=21) was 1.0 SD; after 4 years (N=14), 1.5 SD; and at 5 years (N=9), 1.8 SD. No significant increase in height SDS for bone age was observed. The gain in height SDS over the first year accounted for 30% of the total in over 3 years. The increase in growth velocity was maintained throughout the 3 years, and the height gain over 3 years as compared to the median base line was 5.7 cm. Using multiple regression analysis for the 21 subjects who had completed 3 years of therapy, the authors demonstrated that age combined with birth weight SDS, height SDS, or catch-up SDS significantly predicted 39% of the variance in height SDS over the 3 years. The authors conclude that younger, shorter children have the greatest increase in height SDS, a finding similar to that in children with GH deficiency treated similarly. Further studies are necessary to follow these children to final height in order to demonstrate the effectiveness of GH therapy.

Rakover Y, et al. *Acta Pediatr* 1996;155:851-857.

Editor's comment: *This is a very interesting and important study. Children with Silver-Russell syndrome are often severely handicapped due to both their short stature and low weight to the point that participation in age-appropriate activities is not only difficult but dangerous. Thus, even if final height were not significantly increased by GH therapy, acceleration of growth velocity to achieve a height SDS closer to the normal range at an earlier age would be clearly beneficial to these children. It is interesting to recall that guidelines for GH therapy in New Zealand and Australia include individuals whose height is less than the 1st percentile and growth velocity is less than 25th percentile for bone age and sex. Demonstration of biochemical GH deficiency is not a requirement for therapy in these countries. Thus, these investigators have the opportunity to gather therapeutic information on the effects of GH in a variety of different clinical syndromes and situations that may not be possible in other parts of the world. They should be encouraged to exercise extreme care in the collection of their anthropometric data and in their presentation of such data. It would have been preferable in this particular study to have seen mean and standard deviations rather than median data.*

Previous data on children with Silver-Russell syndrome treated with GH have shown variable results, such as no gain in height SDS for chronologic age after 1 year of therapy or no significant change in height SDS for bone age. Thus, it would be interesting to determine how the individuals identified in the OZGROW data base may be different from those reported in other studies.

William L. Clarke, MD

In Future Issues

Molecular Genetics of Human Chondrodysplasias
William A. Horton, MD

Insulin, the IGF System, and IDDM
Cheri Deal, MD

GH Secretagogues
Allen W. Root, MD

Safety and Effectiveness of Human Growth Hormone Using Pharmacological Dosing
Arnold Slyper, MD

Growth Hormone Increases Breast Milk Volumes in Mothers of Preterm Infants

Recombinant human growth hormone (rhGH) was given for a period of 7 days at 0.2 IU/kg/d (0.53 mg/kg/wk) to a maximum of 16 IU/d to 9 mothers of infants born between 26 to 34 weeks of gestation whose milk production was insufficient to supply their infants' needs. They were compared to 9 mothers of similarly premature infants, who received placebo instead of rhGH (Table 1). The infants of the rhGH-treated mothers were slightly heavier at birth and older at enrollment than those of mothers receiving the placebo. Maternal milk production, measured as the volume of milk obtained by 5 to 6 breast expressions a day and by the gained weight of the infants after breast feedings, increased significantly from 139 ± 49 mL/d at baseline to 175 ± 46 mL/d at 7 days (31% increase) in the rhGH-treated mothers. No significant increase was observed in the placebo-treated mothers, whose baseline production was 93 ± 50 mL/d and rose to 102 ± 69 mL/d at day 7 (7.6% increase). All 9 rhGH-treated mothers had an increase in milk production, whereas 4 of the placebo-treated mothers had a decrease (Figure 1). Insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 increased only in the rhGH-treated group. No adverse effects were seen in the rhGH-treated mothers or their infants. The authors conclude that rhGH treatment in mothers with lactational insufficiency can modestly improve breast milk volumes, although infants' milk needs could be met only with supplementary feeding even when rhGH was used.

Gunn AJ, et al. *Pediatrics* 1996;98:279-282.

Editor's comment: *This paper elicits an interesting concept and expands the currently growing list of uses of rhGH in humans. All pediatricians agree that human milk is best for babies; however, lactation failure continues to be the main cause for its early termination. Nevertheless, clinicians should not immediately prescribe rhGH for the treatment of mothers who do not have sufficient milk production. Sucking and appropriate breast-feeding technique are the single most efficient methods for stimulation of breast milk production. In premature babies who are unable to perform effective suction and, hence, are at increased risk for early termination of maternal milk feedings, mechanical devices to express milk from the mother have been very helpful. When this resource is not successful, a short period of rhGH treatment for the mother may be preferable to a switch to cow's milk-based formulas. The less than optimal matching between babies of rhGH- and placebo-treated mothers, ie, larger and older babies in the rhGH-treated group, could have introduced a bias in the generation of results. An increased production of breast milk could be theoretically expected from mothers of bigger babies independently from the use of rhGH. Further studies with better infant matching need to be performed to prove this efficacy of rhGH.*

Fima Lifshitz, MD

Figure 1
Percent Increase in Milk Volume

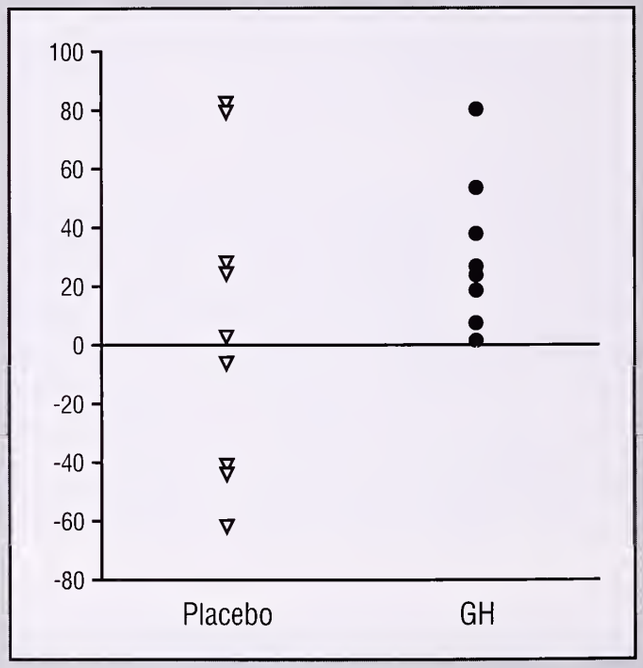

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Table 1
A Comparison of Study Groups Receiving Either hGH or Placebo for 1 Week

	Birth Weight (g)	Gestational Age (weeks)	Age (days)	Weight of Infant (g)	Mothers Age (y)
hGH	1398 ± 397	30.6 ± 3.2	39.7 ± 32	2206 ± 455	32.4 ± 3.6
Placebo	1239 ± 552	30.1 ± 3.2	31.3 ± 18.9	1576 ± 661*	35.7 ± 4.6

*$P<.05$
Reproduced by Permission of *Pediatrics*, Vol. 98, Pages 279-282, Table 1, Copyright 1996.

The New Genomics: Global Views of Biology

The Human Genome Project is well along—by some accounts, ahead of schedule—making it highly likely that the entire human genome will be sequenced by the year 2005. This has led many in the genetics community to ask: "What will be done after this goal is attained?" In the recent Genome issue of *Science*, one of the leaders in this field, Eric Lander, has addressed the question by putting forth several specific goals for what he calls "the new genomics."

First and importantly, Lander views the genome project as the biologist's equivalent of the periodic table. Just as the periodic table gave chemists and physicists building blocks to understand their 19th-century world, the genome project will provide scientists of the next century the building blocks to understand biology. Accordingly, he proposes 10 goals for the next phase of genomics, which he sees as a transition from structural to functional genomics.

1. Routine resequencing of large regions of the human and mouse genome. The rationale is that this will be needed to fully define the extent of variation, eg, polymorphism, in the human genome. Such information will be necessary to understand how genetic variation contributes to the causation of common diseases.
2. Systematic identification of all common variants in human genes. This represents an extension and ordering of the previous goal.
3. Rapid sequencing of other organisms. Lander argues that comparative DNA sequencing will unlock evolutionary relationships not previously appreciated. He notes that sequence conservation will provide a powerful tool to determine functional constraints of genes and their products and a means to identify regulatory regions and important structural features of proteins.
4. Simultaneous monitoring of the expression of all genes. This is needed to generate a complete picture of the state of a cell and a basis for distinguishing among many different cell types. This goal would be achieved through description and cataloging of cell- and tissue-specific gene expression.
5. Develop generic tools for manipulating cell circuitry. Lander reasons that monitoring gene expression is insufficient to understand biologic functions. Rather, he points out that they must be disrupted and manipulated to fully define them; and he urges improvements of tools to accomplish this in model organisms in which functions can be monitored.
6. Monitor the level and modification state of all proteins. This goal focuses on gene products—proteins—rather than genes themselves. Lander argues that many functions of proteins reflect posttranslational modifications, which cannot be determined by analysis of the gene alone.
7. Systematic catalogs of protein interactions. Proteins do not function in a vacuum. Rather, their functions reflect interactions with other molecules; and diseases are due to disturbances in these interactions. This goal would lead to a comprehensive "interaction map" of the genome.
8. Identification and cataloging of basic protein shapes. This represents a more complex level of defining proteins.
9. Increased attention to ethical, legal, and social issues. This goal addresses the need to use the new knowledge in a responsible way.
10. Public education. This is related to goal 9. Lander emphasizes that the new information will provide people with choices regarding how the information will be used. He holds that education is the best safeguard to prevent its misuse.

Lander ES. *Science* 1996;274:536-539.

Editor's comment: *This is a thoughtful and insightful commentary that is useful for both geneticists and nongeneticists alike. It underscores that the Human Genome Project is not an end in itself, but a stepping stone to a more complete understanding of biology and disease.*

William A. Horton, MD

2nd Editor's comment: *Dr. Lander's proposals to study gene aspects are in the broadest perspectives of development, physiology, and microbiology—and are mind-boggling. The analogy that comes to my mind is: "We now have the land, what are we going to build?" The important question to be asked is: "What will all this constructively mean to the human race?" The answers are unknown, but very exciting prospects exist.*

Robert M. Blizzard, MD

Insulin-Like Growth Factor-1 and IGF Binding Protein-3 Remain High After GnRH Analogue Therapy in Girls With Central Precocious Puberty

Kanety et al measured estradiol, insulin-like growth factor 1 (IGF-1), and IGF-binding protein 3 (IGFBP-3) prior to and after 1, 2, and 3 months of GnRHa (D-Trp^6-GnRHa, Decapeptyl 3.75 mg, Ferring, Malmo, Sweden) in 10 girls, aged 7 to 8 years, with central precocious puberty (CPP). Results were compared to those from 7 prepubertal girls aged 8 to 10 years, with no known endocrine abnormalities. The results were analyzed using the nonparametric Wilcoxon signed rank tests and the Pearson χ^2-test. The values were expressed as mean ± SEM.

Pretreatment estradiol levels were in the pubertal range in all 10 patients, fell to levels below the detection limits 1 month after therapy, and remained depressed. Serum IGF-1 was significantly higher in CPP patients as compared to controls (48.8 ± 6.5 nmol/L vs 23.1 ± 4.9 nmol/L; $P < 0.01$). Although serum IGF-1 levels decreased after 1 injection of GnRHa, the decrease was not significant and no further decrease was noted after 2 to 3 months. The changes in IGF-1 levels, when analyzed individually, were heterogeneous and did not follow a specific pattern. Serum IGFBP-3 levels were also significantly higher in CPP patients than in controls (4.70 ± 0.37 mg/L vs 3.71 ± 0.42 mg/L, $P < 0.01$) at baseline and did not change significantly after 1, 2, or 3 months of GnRHa therapy. IGFBP-3 levels were also heterogeneous and did not fall into any specific category. Interestingly, IGF-1 and IGFBP-3 failed to correlate before or during the 3 months of therapy.

The authors review previous studies of the effects GnRHa in CPP on growth hormone (GH), IGF-1 and IGFBP-3. Variable results have been reported, including a decrease in basal and GRF-stimulated GH levels after 3 months of GnRHa therapy without a decrease in serum IGF-1, and a decrease in IGF-1 only after a year of therapy. The authors conclude their data suggest that while estradiol has a role normally in increasing GH, IGF-1, and IGFBP-3, it may not be important in maintaining the levels of these hormones once the increases over the prepubertal state have been established. They speculate that the lack of change in IGF-1 and IGFBP-3 "with GnRHa therapy which has been proven to reduce growth velocity underscores the known effect of sex steroids in the growth process."

Kanety H, et al. *Clin Endocrinol* 1996;45:7-12.

Editor's comment: *This is an interesting article. When one looks at individual data, it would appear that 7 out of the 10 girls had reductions in IGF-1 by 3 months of therapy while 6 out of 10 had reductions in IGFBP-3 during the same period. The lack of consistent patterns and the fact that some girls' levels rose probably accounts for the inability of the data to be significant. It may be that statistical significance could be obtained by studying a larger number of girls. However, the speculation of the authors remains provocative. Should these findings be verified in studies of larger numbers of girls? This may account for the observation that bone age advanced in girls with CPP treated with GnRHa despite adequate suppression of gonadotropins. In addition, this may account for some of the lack of success in achieving significantly greater final heights in children treated with exogenous GH simultaneously with GnRHa therapy.*

William L. Clarke, MD

Increased Energy Expenditure in Growing Adolescents With Crohn's Disease

Growth failure is common in children and adolescents with Crohn's disease and may be the result of reduced energy intake, impaired absorption, or protein-losing enteropathy. In addition, patients with Crohn's disease may have increased energy requirements related to the increase in metabolic activity of their inflamed tissue. Zoli et al measured resting energy expenditure via indirect calorimetry in adolescents with inactive Crohn's disease, both those who were growing and those who had completed their growth. In addition, a control group of healthy growing adolescents was studied. Ten growing adolescents with inactive Crohn's disease (aged 17.8 ± 1.4 years) and 9 who had ceased growing matched for disease, site, and duration (aged 19.0 ± 1.3 years) participated. Subjects had to have histologically proven Crohn's disease with onset prior to age 16, and to have been diagnosed a minimum of 2 years. Height was assessed every 3 months, and those whose height had increased by 2 cm or more during the previous 12 months were considered growing. Nutritional status was assessed by anthropometric measurements from which body mass index, percent body fat, and free fat mass were calculated. Food intake was assessed by 7-day food diary. No subjects were currently receiving corticosteroids and both interleukin 6 and C-reactive protein levels were in the normal range for all subjects.

Resting energy expenditure per kilogram of body weight was significantly higher in growing patients compared to disease controls (32.1 ± 1.6 kcal vs 27.6 ± 0.9 kcal; $P < 0.05$) or healthy controls (24.5 ± 1.0 kcal; $P < 0.001$).

Similar relationships held when resting energy expenditure was expressed per kilogram of fat-free mass. Dietary intake in 5 of the growing patients averaged 97% of the amount recommended for age, sex, weight, and physical activity. Thus, the growing adolescents with inactive Crohn's disease had increased energy expenditure as compared to healthy growing adolescents and nongrowing subjects with inactive disease. The cause of this increase in resting energy expenditure is unknown but could be the result of subclinical disease activity. The authors conclude that nutritional therapy should be directed towards increasing energy intake in these subjects to maximize growth potential.

Zoli G, et al. *Dig Dis Sci* 1996;41(9):1754-1759.

Editor's comment: *The abnormal resting energy expenditure demonstrated in these individuals with inactive Crohn's disease suggests that the metabolic implications of this disease are not necessarily quiescent when the disease becomes clinically inactive. As pointed out by the authors, growth retardation may be the sole manifestation of Crohn's disease in approximately 5% of patients. Until we understand more about the pathophysiology of Crohn's disease, especially in its quiescent state, it may not be possible to suggest alternatives to increased nutrient intake as a means of improving growth in these individuals. Whether antibiotic agents could be of benefit remains to be shown. For further reading regarding management of growth failure in Crohn's disease, see J.A. Walker-Smith's article, "Management of growth failure in Crohn's disease," in* Archives of Disease in Childhood *1996; 75(4):351-354.*

William L. Clarke, MD

Shortened and Diminished Pubertal Growth in Boys and Girls Treated for Acute Lymphoblastic Leukemia

The longitudinal patterns of growth and sexual maturation in 11 Dutch boys and 17 Dutch girls with acute lymphoblastic leukemia (ALL) treated before age 7 years with chemotherapy (including vincristine, prednisone, asparaginase, mercaptopurine, intrathecal methotrexate, and prednisone) and cranial irradiation (24 Gy) were studied. The mean age of onset of the pubertal growth spurt in girls (8.9 ± 0.2 years) was significantly less than that of the (Swiss) reference group (9.7 ± 1.0 years), as were the ages at peak height velocity (10.6 ± 0.7 years vs 12.2 ± 0.8 years); the age at the end of the pubertal growth spurt (12.1 ± 0.8 years vs 13.8 ± 0.8 years); and the duration of the pubertal growth spurt (3.2 ± 0.9 years vs 4.1 ± 0.5 years). This pattern led to a decrease in the pubertal height gain of the ALL girls (20.9 ± 5.3 cm vs 24.7 ± 2.6 cm) and a lower final height (160.8 ± 5.8 cm vs 165.3 ± 5.8 cm). In comparison to normal Dutch females, girls with ALL were younger (10.6 ± 0.71 years vs 11.9 ± 0.84 years) and shorter (146.1 ± 7.5 cm vs 152.8 ± 5.6 cm) at peak height velocity and younger (12.0 ± 0.4 years vs 13.2 ± 0.4 years) and shorter (152.5 ± 3.0 cm vs 162.5 ± 4.1 cm) at menarche, but achieved similar postmenarchal growth (7.1 cm). In comparison to normal Dutch girls with early pubertal onset, girls with ALL were younger (10.3 ± 0.6 years vs 10.7 ± 0.3 years), shorter (144.7 ± 5.7 cm vs 151.8 ± 3.5 cm), and had decreased growth (8.0 ± 0.6 cm/y vs 9.2 ± 1.4 cm/y) at peak height velocity. For males with ALL there were no differences relative to (Swiss) control subjects for age at onset of puberty (11.2 ± 0.7 years), peak height velocity (13.7 ± 0.6 years), or end of the pubertal growth spurt (15.0 ± 0.7 years), but the duration of the pubertal growth spurt was shorter (3.8 ± 0.3 years vs 4.5 ± 0.6 years). Pubertal height gain was less in ALL males (25.9 ± 3.1 cm vs 28.8 ± 4.0 cm) as was final height (170.8 ± 7.6 cm for ALL males vs 177.5 ± 6.7 cm for Swiss males vs 182.0 ± 6.7 cm for Dutch males). In both boys and girls, skeletal maturation progressed more rapidly during puberty than in control subjects. The authors concluded that in children successfully treated for ALL, not only was there a deceleration in growth during treatment, but the pubertal growth spurt was attenuated as well.

Groot-Loonen JJ, et al. *Acta Paediatr* 1996;85:1091-1095.

Editor's comment: *These data should prove a valuable resource with which to determine the effect of intervention (eg, administration of growth hormone, delay of pubertal onset with gonadotropin hormone releasing hormone agonists or antagonists, or dual therapy) on the growth of children who have survived therapy for ALL.*

Allen W. Root, MD

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Instructions: The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. The most common indication for limb lengthening is leg length inequality of:
   a. ≥3 cm
   b. ≥4 cm
   c. ≥5 cm
   d. ≥7 cm
   e. ≥10 cm
2. The Ilizarov method of limb lengthening was an improvement over prior osteotomy and rapid bone distraction methods because of all of the following *except*:
   a. It promoted new bone formation in the distraction gap.
   b. It eliminated complications of joint stiffness and joint dislocation.
   c. It encouraged weight-bearing and patient activity throughout treatment.
   d. It eliminated the need for bone grafting and plate application.
3. Limb length inequality is commonly associated with all but one of the following conditions:
   a. Noonan syndrome
   b. Ollier's disease
   c. Conradi-Hünermann chondrodysplasia punctata
   d. Fibrous dysplasia
   e. Neurofibromatosis
4. Limb lengthening in short stature is controversial because:
   a. It may negatively impact on ultimate joint function and cause early osteoarthritis.
   b. There is little data in seemingly appropriate candidates, such as in patients with metaphyseal dysplasias or Turner syndrome.
   c. Some popular limb lengthening techniques encourage loss of independence due to non–weight-bearing treatment requirements.
   d. Ultimate functional outcomes and the effects on limb growth have not adequately been evaluated.
   e. All of the above
5. Which of the following statements is/are true?
   a. Slow bone formation in fibrous dysplasia limits the utility of limb lengthening.
   b. Despite abnormal fragility of the bone in Sillence types I and IV osteogenesis imperfecta, limb lengthening is appropriate if approached cautiously.
   c. Intrinsic knee instability in congenital limb hypoplasia syndromes precludes the use of limb lengthening to a great extent.
   d. Amputation is indicated for management of severe fibular hemimelia and tibial aplasia.
   e. b and d

Answer Key: 1. C 2. B 3. A 4. E 5. E

CME Accreditation Statement

The University of Virginia School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education activities for physicians.

UNIVERSITY OF VIRGINIA HEALTH SCIENCES CENTER
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Drs. Stanitski, Lifshitz, Clarke, Horton, and Hall report no conflicts; Dr. Root serves on Genentech's National Cooperative Growth Study (NCGS) Advisory Committee; Dr. Blizzard is the President of The Genentech Foundation for Growth and Development which functions independently of Genentech, Inc.

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GROWTH
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Growth Hormone Secretagogues: Physiology and Function

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INTRODUCTION

The first growth hormone-releasing peptides (GHRPs) were engineered by Bowers and colleagues in 1976 while studying the growth hormone (GH)-releasing effect of the endogenous pentapeptide opioid enkephalin and its synthetic analogues. The initial GHRPs were active only in vitro.[1] By systematically alterating amino acid composition, researchers developed the first compound that stimulated GH release both in vivo and in vitro in 1980. It was termed GHRP-6 because it was a hexapeptide. Subsequently, other GHRPs were prepared and denoted by the order in which they were identified (Table 1). Note that GHRP-6 and hexarelin are very similar.

In 1982, Guillemin et al determined the structure of hypothalamic GH-releasing hormone (GHRH), a 44 amino acid polypeptide that stimulates the synthesis and secretion of GH through activation of a guanine triphosphate (G_s-protein) receptor, and consequent increase in somatotroph cytosolic levels of cyclic adenosine monophosphate (cAMP) and Ca^{++}.[2,3] The identification of GHRH resulted in its becoming the focus of research for the next several years.

In 1989, GHRP-6 was demonstrated to release GH. Utilizing structure-function data derived from analysis of GHRP-6 and the concept of a common "privileged structure" that permits specific chemical units to interact with diverse G-protein receptors, Smith et al in 1993 developed nonpeptidyl compounds that simulated the 3-dimensional spatial configuration of GHRP and had GH-releasing activity.[4-6] In 1996, these workers identified the endogenous $G\alpha_{11}$ membrane receptor for these GH secretagogues that activates phospholipase C (PLC).[7,8] These data demonstrate the presence of an endogenous GH-regulating mechanism distinct from GHRH, although the endogenous ligand for this system and its site of origin have not as yet been described. In this presentation, we will review the chemistry and physiology of GHRP and nonpeptidyl GH secretagogues (GHSs) and assess their clinical potential.

CHEMISTRY OF GHRP AND NONPEPTIDYL GH GHSs

Table 1 presents the amino acid structures of several GHRPs in comparison to native met-enkephalin. These compounds were identified sequentially and

In This Issue

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Table 1
Growth Hormone-Releasing Peptides

Amino acid No.	1	2	3	4	5	6	7
Met-Enkephalin	Tyr	Gly	Gly	Phe	Met-NH_2		
GHRP-6	His	DTrp	Ala	Trp	DPhe	Lys-NH_2	
GHRP-1	Ala	His	DβNal	Ala	Trp	DPhe	Lys-NH_2
GHRP-2	DAla	DβNal	Ala	Trp	DPhe	Lys-NH_2	
Hexarelin	His	DMeTrp	Ala	Trp	DPhe	Lys-NH_2	

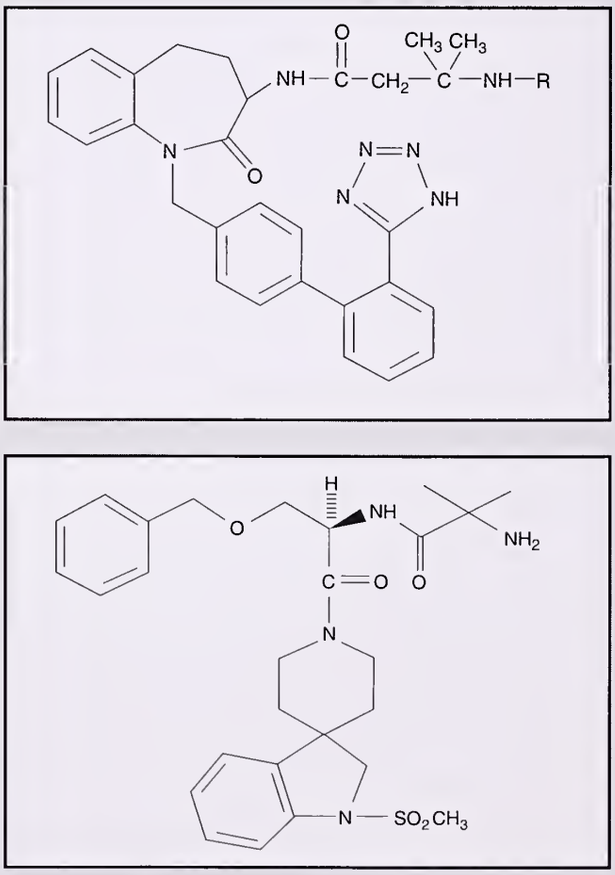
DβNal = D-2-naphthylalanine
DMeTrp = D-methyl-tryptophan

have increasing potency (GHRP-2 > GHRP-1 > GHRP-6 = hexarelin). Figure 1 depicts 2 nonpeptidyl GHSs, L-692,492, a benzolactam, and L-163,191 (MK-0677), a spiropiperidine, that have been most extensively studied. However, tetrahydroquinolone and isoindoline chemical structures with GH-releasing activity also have been designed.[9] Appreciating the relationship of these apparently very different chemical classes of compounds when depicted in 2 dimensions is difficult. However, computer-generated structural overlay maps clearly demonstrate their similarity in 3-dimensional conformation and, hence, their comparable biologic activity. The nonpeptidyl GHS MK-0677 is more potent and has a longer duration of action and greater oral bioavailability than do the GHRPs.

BIOLOGIC ACTIVITY OF GHRPs AND NONPEPTIDYL GHSs

The effects of GHRPs and nonpeptidyl GHSs are similar, as would be anticipated since both groups of secretagogues utilize the same receptor.[7] GHRPs and nonpeptidyl GHSs stimulate the secretion of GH in vitro and, in a variety of species, in vivo, including humans. They are active when administered intravenously, intramuscularly, subcutaneously, intranasally, and orally. In normal, short-statured, and obese children and in normal young and elderly adults, GHSs stimulate greater secretion of GH in vivo than does GHRH, but do so only in the presence of endogenous GHRH. The GH-releasing effects of combined GHRH and GHRP are synergistic. As with GHRH, the GH-releasing activity of GHSs is inhibited by somatostatin (SRIH). The amplitude, but not the frequency, of GH pulses is increased when GHSs are continuously infused over 24 to 36 hours; the acute GH secretory response to GHSs is attenuated, but that to GHRH is preserved.[1] These observations suggest that an as yet uncharacterized endogenous GHS influences the amplitude of GHRH-induced GH secretion. Similarly, the continuous infusion of GHRH also increases GH pulse amplitude and desensitizes the pituitary to an acute bolus of GHRH but not to GHS. Thus, these GH secretagogues induce homologous but not heterologous desensitization. In prepubertal children, the GH-releasing effects of GHSs are reproducible and enhanced by

Figure 1



Chemical structures of 2 nonpeptidyl GHSs. L-692,429 (top) is a benzolactam. L-163,191 (MK-0677) is a spiropiperidine (bottom).

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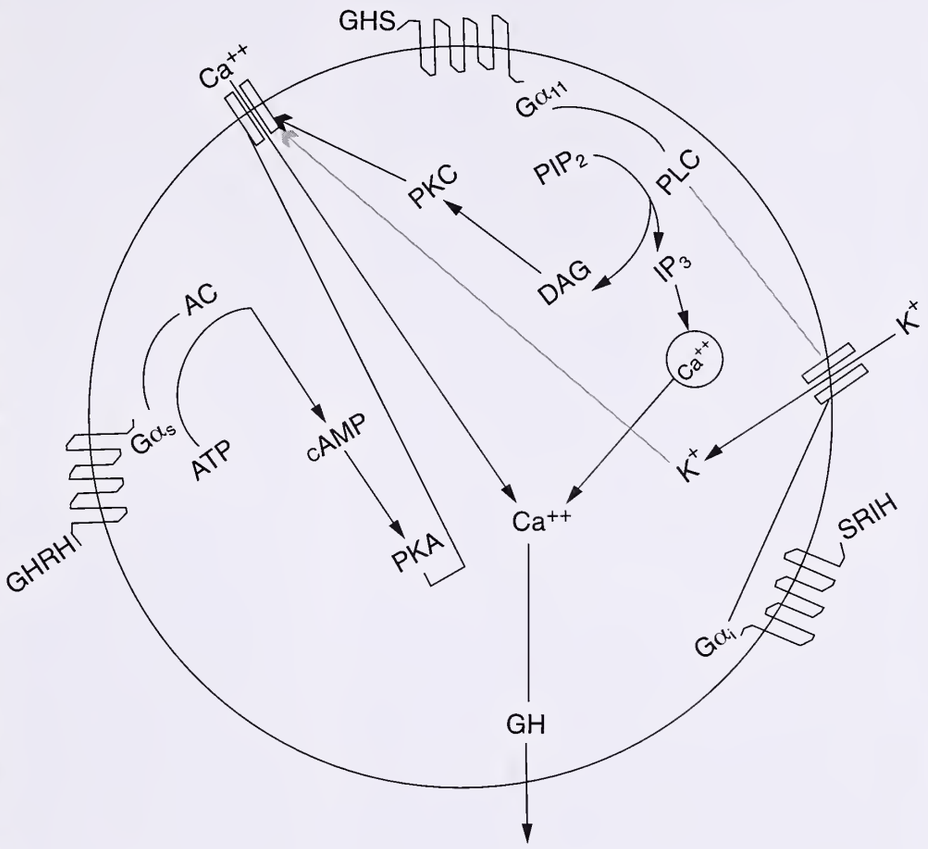
Figure 2



Cellular action of GH releasing and GH-inhibiting agents. Acting through cAMP, GHRH increases PKA activity, leading to phosphorylation of L-type Ca^{++} channels and increased transport and IC Ca^{++}. SRIH increases transport of K^+, thus raising IC levels of K^+ and inhibiting Ca^{++} transport. GHS increases IC Ca^{++} by activating PLC that (1) inhibits K^+ transport, (2) mobilizes Ca^{++} from calciosomes through IP_3, and (3) enhances Ca^{++} transport through DAG activation of PKC. GHRH (GH-releasing hormone); GHS (GH secretagogue); SRIH (somatostatin); ATP (adenosine triphosphate); cAMP (cyclic adenosine monophosphate); PKA (protein kinase A); PLC (phospholipase C); PIP_2 (phosphatidylinositol); IP_3 (inositol triphosphate); DAG (diacylglycerol); PKC (protein kinase C); AC (adenylyl cyclase); IC (intracellular). Solid line represents (stimulatory effect); dotted line represents (inhibitory effect).

Adapted with permission from Smith RG, et al. Mechanism of action of GHRP-6 and nonpeptidyl growth hormone secretagogues. In: Bercu BB, Walker RF, eds. *Growth Hormone Secretagogues.* New York, NY:Springer-Verlag;1996:147-163.

pretest priming with estradiol or testosterone but not with oxandrolone.[10]

GHSs do not stimulate secretion of thyrotropin, luteinizing hormone, or follicle-stimulating hormone, but variably increase serum concentrations of prolactin and cortisol. GHSs stimulate release of GH in most short-statured children with GH deficiency (GHD), except those with interruption of the pituitary stalk or absence of the adenohypophysis.[11-15] In GHD children without evident anatomic insult to the hypothalamic-pituitary unit, GHS-induced GH secretion is quantitatively similar to that of GHRH and is synergistic with GHRH.

THE GHS RECEPTOR

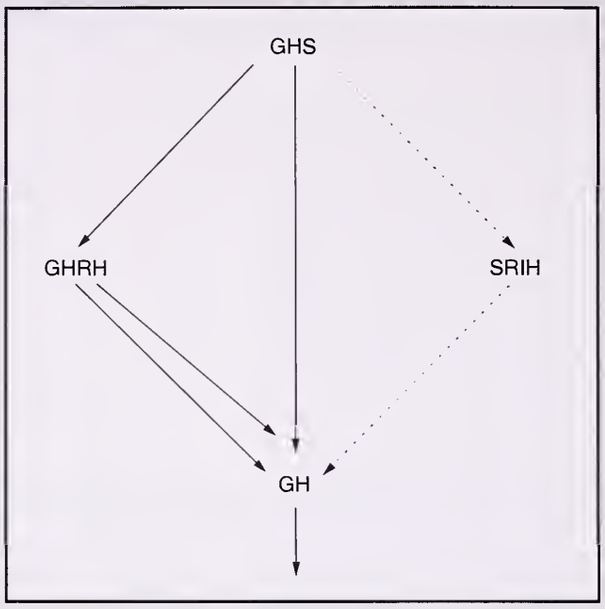
GHRH acts through a 423 amino acid G_s-protein receptor with 7 membrane-spanning domains whose gene is located at human chromosome 7p14. The GHRH receptor primarily activates adenylyl cyclase, leading to increased intracellular (IC) levels of cAMP and protein kinase A; activation of L-type Ca^{++} channels, resulting in increased cytosol Ca^{++} levels; immediate release of stored GH; and subsequent increase in GH synthesis (Figure 2).[16,17] GHS is unable to interact with the GHRH receptor. The functional G-protein receptor for GHS (Ia) is a 366 amino acid polypeptide with 7 transmembrane domains whose gene is located at human chromosome 3q26.2.[7,18] The second, nonfunctional isoform of the GHS receptor (Ib) is formed by alternative mRNA processing and is truncated at 289 amino acids; therefore, after the fifth transmembrane domain it terminates with an IC domain of 58 amino acids. GHS receptor Ib fails to bind or biologically respond to GHS, and its function is unknown.

Messenger RNAs for GHS receptor types Ia and Ib are expressed in very low amounts in both the anterior and posterior pituitary (their levels are 1/100th the receptor number of GHRH and SRIH receptors). They also are expressed in the arcuate, ventromedial, and supraoptic nuclei, infundibular hypothalamus (in neurons near the median eminence), hippocampus, and dentate gyrus.[7,18]

SRIH (acting through several G_i-protein receptors) activates K^+ channels, leading to hyperpolarization and inhibition of Ca^{++} channel function and thereby antagonizing the secretion of GH induced by GHRH and GHS (Figure 2). Through the $G\alpha_{11}$ subunit, type Ia GHS receptor activates PLC, which inactivates K^+ channels, leading to cellular depolarization and activation of Ca^{++} channels. PLC also hydrolyzes membrane phosphatidylinositol, thus increasing cytosol concentrations of inositol triphosphate and diacylglycerol (Figure 2).[8] Diacylglycerol activates protein kinase C (PKC), which also activates L-type transmembrane Ca^{++} channels and increases IC Ca^{++} concentrations and GH secretion. Thus, one of the important functions of the GHS is to antagonize the inhibitory effects of SRIH on GH release stimulated by GHRH. These data explain, in part, the necessity for somatotroph exposure to GHRH in order for the GHS to act—that is, the latter works in large part by permitting GHRH to act. The PLC signal transduction system also can be utilized by GHRH and the adenylyl cyclase system can be activated by GHRP-2.[19]

The presence of GHS receptors in the hypothalamus indicates that these agents also act centrally (Figure 3). Indeed, this may be their primary site of action, as they increase electrical activity and c-*fos* expression in GHRH and neuropeptide Y-containing neurons within the arcuate nucleus. Levels of GHRH in the pituitary portal vasculature also are increased.[17,20,21] GHS may act directly on GHRH neurons or indirectly through an as yet unknown factor. The dual site of GHS action permits these secretagogues to influence the amplitude of GHRH-mediated endogenous GH secretion. GHSs also stimulate release of arginine vasopressin, accounting for the transient increase in cortisol levels recorded after their administration.[22]

Figure 3



Integrated effects of GHRH, GHS, and SRIH on the regulation of GH secretion. Within the hypothalamus GHS stimulates GHRH release (either directly or through an unknown factor) and perhaps inhibits SRIH release. GHS directly stimulates GH release from the pituitary somatotrope in the presence of GHRH. The solid line represents a stimulatory effect; the dotted line represents an inhibitory action.

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CLINICAL SIGNIFICANCE OF THE GHSs

GHRH and GHSs have been tested to determine if they are of use in diagnosing the presence and/or the etiology of GHD. Since the most reliable way to identify the GHD patient with an anatomically intact hypothalamic-pituitary unit—and without a known insult to the central nervous system—is unclear,[23,24] the role of GHS testing in this process has not been defined. Bercu and Walker[25,26] proposed a scheme to distinguish between deficiency of GHRH, deficiency of endogenous GHS, and deficiencies of both by sequential administration of GHRH and GHS. Its utility has yet to be verified.

Mericq et al[13] reported that 15/22 children with idiopathic GHD responded to an acute injection of GHRH with a significant increase in GH levels; 12/22 responded to GHRP-1; a total of 19/22 responded to a combination of the 2 agents. Five children did not respond to either secretagogue alone, but did respond to the combination of agents. Three children did not respond to any single or combined stimuli. Nineteen other children with clinical and hormonal findings consistent with idiopathic GHD have had normal GH secretion in response to GHRH and GHS.[12,14] These data confirm the difficulty in establishing the diagnosis of idiopathic GHD and assigning its pathophysiology to an absence of either GHRH or the endogenous secretagogue. Although a positive GH secretory response to GHS implies the presence of somatotropes and their exposure to GHRH, an absent or blunted response does not identify the site of error in GH secretion. Thus, testing with GHS cannot replace more standard studies that primarily establish the presence of deficient GH secretion in the appropriate clinical setting.

GHRH and GHSs have been and are being investigated as therapeutic agents. GHSs could be useful therapeutically only in patients with the ability to secrete GH in response to GHSs. Laron et al[27,28] administered hexarelin intranasally to 8 prepubertal, short, normal children for 8 to 10 months and recorded an increase in growth velocity from 5.3 to 7.4 cm/y with a parallel increase in bone maturation. After hexarelin was discontinued, growth rate stabilized or declined. Mericq et al[29] treated 6 GHD children with subcutaneous GHRP-2 for 6 months, and observed an increase in mean growth rate from 2.5 to 5.6 cm/y. Pihoker et al[30] administered GHRP-2 intranasally to 15 GHD children for 3 to 4 months and recorded an increase in mean growth velocity from 3.6 to 6.7 cm/y. In neither study did IGF-1

levels increase during treatment. Since these studies were of extremely brief duration, the effects of more prolonged administration of GHSs to children with GHD need to be studied to establish the role of GHSs in the management of this disorder. Whether the combined use of GHRH or its analogues or GHSs will be useful in the treatment of some GHD subjects is unknown at this point, but is a potential direction for further research. Given the disappointing results of GH treatment of normal short children,[31] it is unlikely that GHSs alone will prove effective in this group of children.

Consideration is being given to the therapeutic usefulness of GHRH and/or GHSs in older adults. GH secretion wanes between the third and fifth decades of life, possibly due to increase in somatostatinergic tone. Thorner[32] suggested alternatively that the decline in GH secretion with aging may be related to a decline in production of endogenous GHS with ensuing decreases in GHRH and GH secretion. In healthy elderly subjects, the GH secretory pulse amplitude can be increased by administration of GHRH or a GHS intravenously or orally.[32-34] Bach[33] reported that in elderly subjects with basal levels of IGF-1 <165 ng/mL, oral administration of 25 mg of MK-0677 for 6 months increased mean 24-hour GH concentrations, serum levels of IGF-1, and lean body and fat mass, and prevented decline in shoulder and knee strength when compared with placebo-treated subjects. Whether therapy with GH, GHRH, and/or a GHS will have long-term beneficial effects on the quality of life of the elderly subject without undue adverse effects has yet to be determined. If GH proves of value in the management of surgical wounds, burns, fractures, heart failure, or debilitating diseases such as AIDS, GHSs also might be useful in the management of these problems.[35]

FUTURE DIRECTIONS

Future efforts will be focused on identifying the endogenous ligands that form this new system of GH regulation, such as localizing the cellular site of endogenous GHS production; determining the mechanisms by which its secretion is controlled; defining the physiologic role of GHS in normal and pathologic situations; establishing procedures to identify deficiency of the endogenous ligand; and determining the diagnostic and therapeutic utility of exogenous GHS in children with growth retardation, the elderly, patients with debilitating illnesses, and other appropriate clinical situations. Much illuminating investigation remains to be done.

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CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimal clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

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Tyrosine Kinases: Their Role in Producing Endocrine and Other Cancers

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The history of tyrosine kinase oncogenes can be traced to 1911 when Francis Peyton Rous, of the Rockefeller Institute, demonstrated that a chicken tumor could be transplanted using a cell-free filtrate. Thus, the field of tumor virology was born. However, it was not until more recent advances in molecular biology that it was recognized that the Rous sarcoma virus transforms cells due to the presence of a tyrosine kinase, the *src* oncogene, in its genome. In 1976, Drs. Harold Varmus and J. Michael Bishop published the finding that the *src* oncogene in the virus was not a true viral gene but instead was a normal cellular gene that the virus had acquired during replication in the host cell. These normal cellular homologues are referred to as proto-oncogenes. However, the transforming version of the *src* oncogene was mutated, and constitutively active, as compared with the tightly regulated normal cellular SRC protein tyrosine kinase.

Since the discovery of the *src* oncogene as a tyrosine kinase, numerous tyrosine kinases have been found, and now number more than 100. Tyrosine kinases can be divided into 2 primary categories: receptor and nonreceptor tyrosine kinases (Figure 1). Receptor tyrosine kinases, such as the insulin receptor and the insulin-like growth factor receptor, contain extracellular ligand binding domains that bind to specific proteins, such as hormones or growth factors.[1] Ligand binding induces activation of the intracellular tyrosine kinase domain, leading to the initiation of signaling events specific for the receptor.

Nonreceptor tyrosine kinases are intracellular cytoplasmic proteins that are linked to a variety of transmembrane receptors, such as the growth hormone or prolactin receptors.[2,3] These nonreceptor tyrosine kinases are similarly activated after binding of ligand to their associated receptors (Figure 1).[2,3]

The list of tyrosine kinases also includes proteins that are involved in pathways that regulate cellular growth, activation, and differentiation. Despite the essential contribution of tyrosine kinase oncogenes to our current understanding of a variety of cellular signaling pathways and their historical importance to the field of tumor biology,[4] only a limited role for tyrosine kinases in human cancers has been identified. This article reviews the mechanisms by which protein tyrosine kinases may participate in malignant transformation and the tyrosine kinases that are known to play prominent roles in the production and growth of human cancer.

ONCOGENIC ACTIVATION OF TYROSINE KINASES

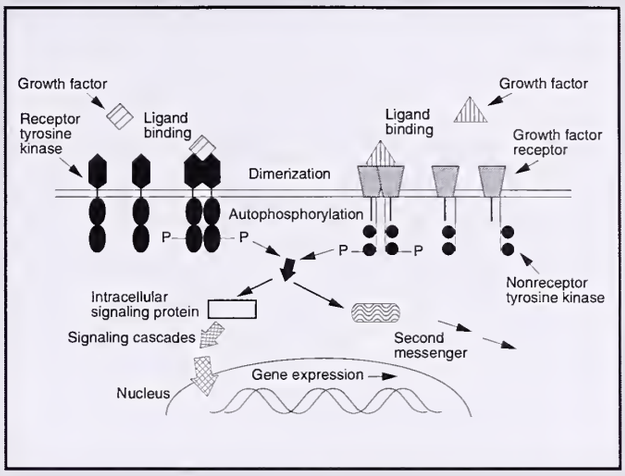
Several mechanisms by which a tyrosine kinase might acquire transforming function exist, but in all cases the result is constitutive activation of a protein that is normally tightly regulated. To understand how constitutive activation of a tyrosine kinase might occur, the normal pathways that regulate kinase activity require review (Figure 1).

When a growth factor binds to its receptor, the receptor or its associated tyrosine kinase becomes transiently activated, which leads to the activation of other proteins in the growth-stimulatory pathway and the consequent production of small regulatory molecules called second messengers. These signals are ultimately transmitted to the nucleus, where expression of specific genes is induced and lead to cell division. At the same time, growth-inhibitory signals are generated normally to prevent cellular proliferation from continuing indefinitely. One of the functions of these inhibitory signals is the deactivation of the tyrosine kinase. Precise control of these positive and negative signaling events is necessary to maintain normal cellular growth.

Constitutive activation of tyrosine kinases occurs by several mechanisms (Figures 2 and 3). The first is by overproduction of a growth factor or by concomitant production of a growth factor and its receptor, which leads to abnormal receptor stimulation and activation (Figure 2). A second mechanism is mutation of the tyrosine kinase, which leads to constitutive activity. Mutations may occur in either the catalytic or the regulatory regions of the protein, or mutations can occur in proteins that regulate the activity of tyrosine kinases (Figure 3).

Two tyrosine kinases have been clearly implicated in human diseases. These two, RET (REarranged during Transfection)[5] and ABL (isolated from the Abelson leukemia virus), are constitutively activated as the result of several different types of mutation. They serve as examples of the potential role of tyrosine kinases in human cancers.

Figure 1
Signaling Through Tyrosine Kinases



Signaling through receptor (left) and nonreceptor (right) tyrosine kinases.
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MULTIPLE ENDOCRINE NEOPLASIA AND THE RET TYROSINE KINASE

Multiple endocrine neoplasia type 2 (MEN 2) and familial medullary thyroid carcinoma (FMTC) are clinically distinct syndromes characterized by a predisposition to the development of endocrine tumors (Table 1). They have in common the occurrence of medullary thyroid cancer, which is the most common cause of death in affected families. Four distinct subtypes of MEN 2 exist: MEN 2A1, 2A2, 2A3, and 2B (Table 1).

FMTC is characterized by the development of bilateral medullary thyroid carcinoma at an average age of 40 to 50 years. To be classified as FMTC, neither patients nor their family members may have pheochromocytoma or parathyroid disease.

In contrast, patients with MEN 2A develop medullary thyroid carcinoma at an earlier age, typically between 20 and 30 years of age. Further, patients with MEN 2A also may develop pheochromocytoma and parathyroid hyperplasia (MEN 2A1), or only pheochromocytoma (MEN 2A2), or parathyroid adenomas or hyperplasia (MEN 2A3).

In Future Issues

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Cheryl Deal, MD

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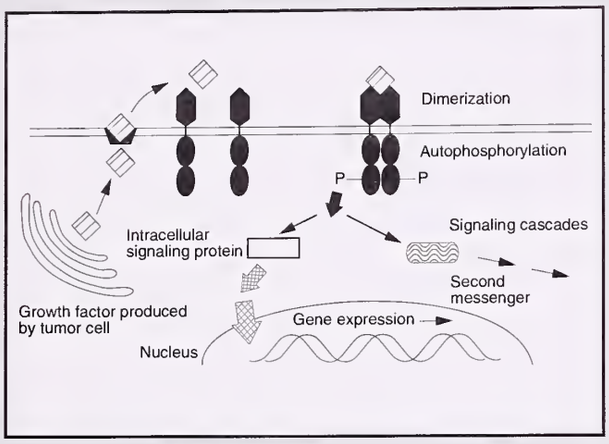
William A. Horton, MD

How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?
Arnold Slyper, MD

The Role of Leptin and Its Receptor in Obesity
Rudolph Leibel, MD

Figure 2
Tyrosine Kinase Activation by an Autocrine Growth Loop



Constitutive ligand production resulting in tyrosine kinase activation.

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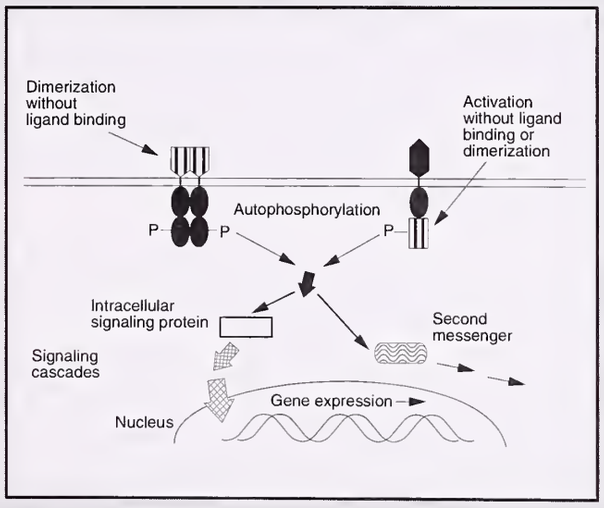
Figure 3
Mechanisms Leading to Constitutive Kinase Activity



Mutations can produce constitutive tyrosine kinase activation. This might occur by a mutation in the extracellular domain (shown on the left) that causes receptor dimerization independent of ligand binding. Another possibility would be a mutation in the cytoplasmic domain that leads to kinase activation without either ligand binding or receptor dimerization (shown on the right).

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MEN 2B has a more complex phenotype. All patients have pheochromocytoma and medullary thyroid carcinoma, but parathyroid involvement is rare. In addition, there usually are consistent developmental abnormalities, including characteristic facies, a marfanoid habitus, thickened corneal nerves, skeletal abnormalities, mucosal neuromas, and sometimes diffuse intestinal ganglioneuromas.

The genes responsible for each of the MEN 2 subtypes were identified using a combination of physical mapping and genetic linkage techniques.[6,7] These disorders were known to be inherited in an autosomal dominant fashion. Initial family studies localized the MEN 2A gene to a region of chromosome 10. Soon thereafter, linkage analysis mapped MEN 2B and FMTC to the same region as MEN 2A, suggesting that mutations in a single gene might be responsible for all 3 clinical phenotypes. Physical mapping of the region on chromosome 10 narrowed the MEN 2 locus to a 480-kb area in which the ret proto-oncogene was mapped, and *ret* emerged as the most likely candidate for the MEN 2 gene. In 1993, germline mutations of *ret* were identified in DNA from MEN 2A patients and in DNA from FMTC patients by 2 independent groups,[8,9] confirming the potential role of the RET tyrosine kinase in these disorders. A map of the RET tyrosine kinase and the mutations associated with specific disease phenotypes is depicted in Figure 4.

The *ret* proto-oncogene encodes a transmembrane receptor tyrosine kinase whose normal function and ligand remain unknown. In the adult, RET is expressed in cells and lineages derived from the branchial arches and neural crest, including the thyroid, parathyroid, adrenal medulla, enteric ganglia, brain, and autonomic nervous system.

The majority of *ret* mutations associated with both MEN 2A and FMTC involve any 1 of 5 cysteine residues located in the extracellular ligand-binding domain of RET. Both mutant and wild-type alleles are retained in tumor DNA, suggesting a dominant mechanism in the development of these malignancies. The most common mutation occurs at cysteine 634, accounting for 74% of *ret* mutations in families with FMTC and with all MEN 2A syndromes; the percentage increases to 92% in families with medullary thyroid carcinoma, parathyroid disease, and pheochromocytoma (MEN 2A1). Although it is clear that *ret* mutations are associated with med-ullary thyroid carcinoma, these data suggest that other factors contribute to the distinct disease phenotypes.

In MEN 2B patients, a single germline point mutation in the cytoplasmic tyrosine kinase domain of *ret* was reported by several groups in 1994.[10,11] About 95% of MEN 2B patients have the same point mutation, substituting a threonine for methionine at codon 918 in the RET tyrosine kinase domain. This mutation has been shown to be inherited with only this MEN disease phenotype and no other. As with MEN 2A mutations, both the wild-type allele and the mutant allele are present in tumor DNA, consistent with the autosomal dominant inheritance.

All the *ret* mutations examined lead to constitutive activation of the RET tyrosine kinase. However, the mechanism of RET activation differs for the mutations commonly associated with MEN 2A and

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GROWTH, Genetics, & Hormones
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1. Educational value of newsletter	5	4	3	2	1
2. Clinical relevance of articles	5	4	3	2	1
3. Newsletter style/format	5	4	3	2	1
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5. Were the educational objectives met?	5	4	3	2	1

6. In your opinion, how could this newsletter be improved?

_____

_____

_____

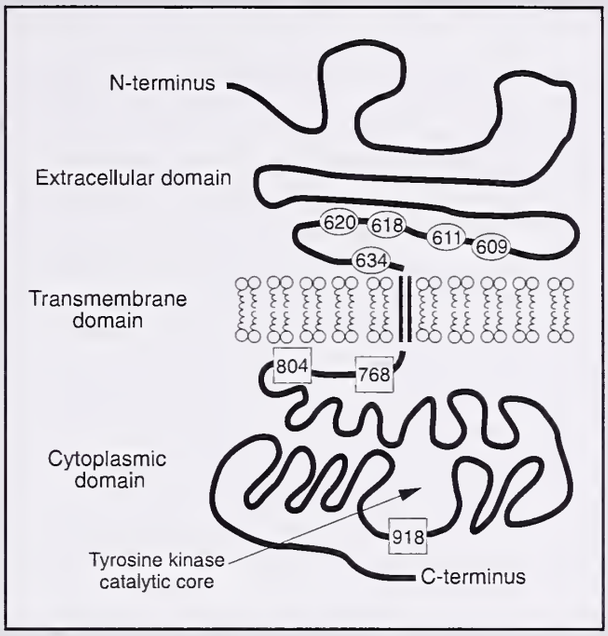
MEN 2B.[7] Normally, upon ligand binding, receptor tyrosine kinases dimerize, autophosphorylate, and as a result are activated. RET tyrosine kinases with amino acid 634 mutations characteristic of MEN 2A undergo dimerization and activation in the absence of ligand. In contrast, the single point mutation of methionine 918 in the tyrosine kinase domain of RET in MEN 2B leads to activation of the RET tyrosine kinase in the absence of dimerization. These 2 different mechanisms of activation suggest a potential explanation for the divergence in disease phenotypes.

Probably *ret* mutations are not sufficient for tumorigenesis in vivo. Cancer is typically thought to be a multistep process, resulting from an accumulation of defects in genes involved in the positive or negative regulation of cell proliferation and survival. As most patients with MEN 2 have thyroid C-cell hyperplasia, it is possible that the inherited activation of the RET tyrosine kinase leads to thyroid proliferation, with additional acquired somatic mutations leading to the development of cancer. If this scenario is correct, one might also expect to see *ret* mutations in sporadic cases of thyroid cancer. In fact, some of the same *ret* mutations seen in familial cases of medullary thyroid carcinoma are present in sporadic cases.[6,7,12] A somatic mutation of methionine 918 has been detected in 30% to 40% of sporadic medullary thyroid carcinomas.[10,11] A mutation at amino acid 768 of *ret*, present in 10% of cases of FMTC, also has been found in some sporadic cases of medullary thyroid carcinoma. Some cases of sporadic pheochromocytomas also contain somatic mutations of methionine 918 or in the MEN 2A region. These somatic mutations are distinguished from familial cases by comparing germline DNA to tumor DNA. In sporadic cases, *ret* mutations are seen in the tumor but not in normal tissues; in familial cases, *ret* mutations are seen in both.

Rearrangements of *ret* also have been found in 10% to 35% of human papillary thyroid carcinomas.[7,12] These rearrangements fuse the RET tyrosine kinase domain to sequences from another cellular protein. This results in a deletion of the extracellular ligand binding domain and leads to a constitutively activated RET tyrosine kinase. This activating RET rearrangement was observed in 60% of cases of papillary thyroid carcinoma in children from areas contaminated by the Chernobyl accident.[13] This same rearrangement of RET occurs in cell lines exposed to in vitro irradiation, suggesting that the RET rearrangement was induced by radiation exposure.[14]

Identification of germline mutations of the *ret* proto-oncogene in MEN 2A, MEN 2B, and FMTC and demonstration of *ret* mutations and rearrangements in sporadic medullary thyroid cancer and papillary thyroid carcinoma distinguishes *ret* as the most important gene involved in the development of thyroid cancer. A data base has been established to maintain and analyze *ret* mutations in MEN 2 families.[15] Discovering correlations of specific mutation

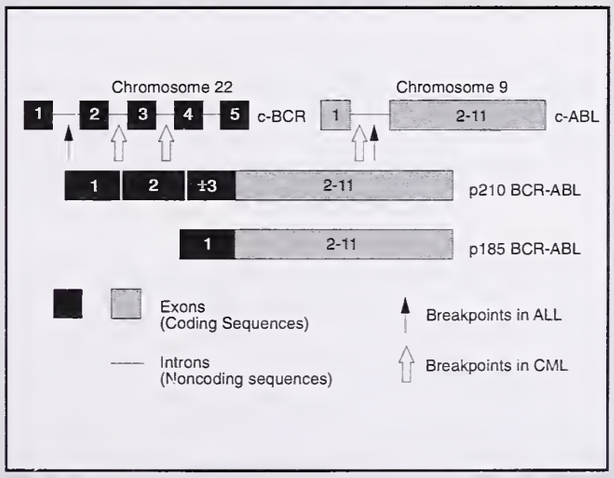
Figure 4
RET Tyrosine Kinase



Schematic of the RET tyrosine kinase. Amino acid mutations frequently observed in MEN 2 in the extracellular domain of RET are portrayed as ovals. Rectangles denote amino acid mutations in the intracellular domain. Gray shaded mutations are found in MEN 2A or familial medullary thyroid carcinoma. The white rectangle depicts the MEN 2B amino acid mutation.

From Goodfellow PJ, Wells SA. RET gene and its implications for cancer. *JNCI.* 1995;87(20):1515-1523.

Figure 5
The Molecular Consequences of the Philadelphia Chromosome Translocation



The Philadelphia chromosome translocation juxtaposes sequences of the breakpoint cluster region (BCR) on chromosome 22 with the gene encoding the c-ABL tyrosine kinase from chromosome 9. The BCR-ABL fusion proteins found in CML and ALL are depicted.

With permission of Druker BJ, et al.

with specific phenotypes would have substantial implications for screening MEN families.[6,15,16] Thus far, for example, mutations at amino acid 768 have been found only in FMTC, and not in any case of MEN 2A or MEN 2B. Data will need to be compiled from many more families to substantiate these findings.

DNA testing can now serve both to confirm the clinical diagnosis and predict the MEN 2 syndrome in individual family members. In the absence of a germline mutation, a patient with isolated medullary thyroid cancer can now be appropriately identified as a sporadic case. In a MEN 2A family, detection of a *ret* mutation in a family member can facilitate appropriate biochemical screening or consideration of prophylactic thyroidectomy, while mutation-negative individuals can be reassured. In MEN 2B, with its associated single germline mutation in amino acid 918, DNA testing offers a significant clinical advantage. Thyroid cancer in MEN 2B can present in childhood; therefore, *ret* mutation testing provides a true breakthrough in screening, providing opportunities for preventive management. Further correlation between mutation location and phenotype may permit streamlined surveillance of MEN 2 patients in the future. DNA testing has proven cost-effective in another inherited cancer syndrome, retinoblastoma, and may be similarly cost-effective in MEN 2.

LEUKEMIA AND THE ABL TYROSINE KINASE

Chronic myelogenous leukemia (CML) accounts for approximately 20% of adult leukemia cases. CML is characterized clinically by an initial chronic phase, during which there are excess numbers of white blood cells in the peripheral blood and bone marrow. During the chronic phase of the disease, white blood cells mature normally and there is a full spectrum of white blood cells, from blasts to neutrophils, circulating in the peripheral blood. After a median of 4 years, there is a transition to an accelerated, or blast, phase. This transition is accompanied by the loss of the capacity for terminal differentiation of white blood cells, resulting in an acute leukemia.

In 95% of CML cases, the Philadelphia chromosome is detectable. This chromosome abnormality is a somatic mutation that occurs in a hematopoietic stem cell as a result of a reciprocal translocation between chromosomes 9 and 22. This balanced translocation fuses sequences of the breakpoint cluster region (bcr) on chromosome 22 with the c-*abl* tyrosine kinase from the long arm of chromosome 9 (Figure 5).[17]

Specific BCR-ABL fusion proteins lead to distinct disease phenotypes. In CML, the BCR-ABL fusion protein contains 927 or 902 amino acids from BCR fused to the ABL tyrosine kinase. This fusion protein, termed p210 BCR-ABL, is found in 95% of patients with CML. However, it is also present in 5% to 10% of adults with acute leukemia for whom there is no evidence of antecedent CML. Another BCR-ABL fusion protein, p185 BCR-ABL, which contains only 426 amino acids from BCR, occurs in 10% of adult cases and 5% to 10% of pediatric cases of acute lymphoblastic leukemia, but is never seen in CML.[18,19] Various BCR-ABL fusion proteins are shown in Figure 5.

The BCR-ABL fusion proteins have severalfold elevation of tyrosine kinase activity over that in the normal c-*abl* kinase,[20] and the tyrosine kinase activity of the BCR-ABL protein correlates with the disease phenotype. For example, p185 BCR-ABL is more active as a tyrosine kinase than p210 BCR-ABL and is associated with a rapidly progressive acute leukemia. The p210 version of BCR-ABL, although activated as a tyrosine kinase as compared to c-*abl*, is less active than p185 BCR-ABL and is associated with a more indolent disease phenotype.

In many in vitro assays of tumorigenicity *bcr-abl* has transforming ability. However, the clearest

Table 1
MEN 2 Subtypes and FMTC Clinical Features

Category	Clinical Features			
	Medullary Thyroid Carcinoma	Pheochromocytoma	Hyperparathyroidism	Other Clinical Features
MEN 2A (1)	yes	yes	yes	
MEN 2A (2)	yes	yes	no	
MEN 2A (3)	yes	no	yes	
MEN 2B	yes	yes	no	Typical facies, mucosal neuromas, skeletal abnormalities, intestinal ganglioneuromas
FMTC	yes	no	no	Late onset, more indolent course

FMTC, familiar medullary thyroid carcinoma; MEN, multiple endocrine neoplasia

evidence for the involvement of *bcr-abl* in leukemia comes from studies from the laboratories of Drs. Owen Witte and David Baltimore. In these studies, investigators expressed *bcr-abl* in murine bone marrow cells and used these cells to reconstitute lethally irradiated mice. These mice develop a syndrome resembling CML as well as other leukemias.[21,22] These data implicate *bcr-abl* as the cause of CML, and provide the strongest evidence for a protein tyrosine kinase as the etiologic agent in a human malignancy.

PROTEIN TYROSINE KINASES AND OTHER CANCERS

Aside from the examples noted above, implication of tyrosine kinases in the etiology of other human malignancies has been difficult. Both *ret* and *abl* were identified in human cancers because of evident genetic abnormalities — heritable predisposition to cancer in the first instance, and characteristic cytogenetic abnormalities in tumor cells in affected patients in the second. These genetic abnormalities led to the discovery of activating mutations in tyrosine kinases and implicated the tyrosine kinase as the cause of the disease. Thus, the contribution of genetic abnormalities and mutations has been strong evidence for the involvement of ABL and RET tyrosine kinases in human diseases. Unfortunately, although most other cancers have associated genetic abnormalities, no other frequent mutations in tyrosine kinases have been found to suggest an etiologic role in a specific tumor.

As previously noted, there are several mechanisms by which tyrosine kinases can become constitutively activated in the absence of mutations in the tyrosine kinases. These mechanisms include autocrine production of a growth factor or mutations in proteins that regulate tyrosine kinase activity. There are numerous examples of possible autocrine growth loops involving tyrosine kinases in many human cancers.[23] However, in the absence of activating mutations, determining their contribution as a cause of the underlying malignancy has been difficult.

Tyrosine kinases also could have important roles in tumor progression. Activated tyrosine kinases might confer a proliferative advantage to subpopulations of cancer cells or they could potentiate tumor metastasis or tumor neovascularization. Expression of certain tyrosine kinases in some human cancers has been shown to convey prognostic significance, supporting the concept that tyrosine kinases may be involved in disease progression. It remains to be determined if tyrosine kinases actually contribute to poor outcomes or are simply a reflection of a general dysregulation of cellular processes associated with advanced cancer. However, given the central role of tyrosine kinases in the control of cellular growth and differentiation, it is likely that tyrosine kinases are involved in many aspects of tumorigenesis.

Identifying molecular abnormalities in human tumors may lead to opportunities for therapeutic intervention. We have recently reported an ABL-specific tyrosine kinase inhibitor that kills BCR-ABL-expressing cells, but not normal cells, in vitro and in vivo.[24] Clinical studies with compounds such as these will be required to validate the concept that specific activated tyrosine kinases in human cancers can be targeted for therapeutic benefit.

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Recombinant Human Growth Hormone and Recombinant Human Insulin-Like Growth Factor 1 in Patients With HIV-Associated Wasting

Previous open-label studies of short duration have demonstrated that rhGH or rhIGF-1 increases body weight and lean body mass and decreases body fat in adults with wasting (> 10% weight loss) associated with HIV infection and AIDS.

Schambelan et al report that in a 12-week, randomized, double-blind, placebo-controlled multicenter study of 178 HIV-infected patients, rhGH (0.1 mg/kg/d; average dose, 6 mg/d) increased body weight (1.6 ± 3.7 kg), lean body mass (3.0 ± 3.0 kg), and total (2.4 ± 3.1 L) and intracellular (1.3 ± 2.9 L) body water, while there were no changes in these values in the placebo group. Body fat declined (-1.7 ± 1.7 kg) in rhGH-treated patients, but did not decrease significantly in the placebo-treated patients. In the rhGH-treated group, treadmill work output increased an average of 13.2% after 12 weeks. The perceived health status or use of health facilities in rhGH-treated subjects did not change. rhGH was reasonably well tolerated, but many patients developed edema, arthralgia, and diarrhea. The authors concluded that rhGH could partially reverse the wasting associated with HIV infection, but this was not accompanied by a subjective improvement or alteration in disease status.

Waters et al conducted a double-blind study of the effect 12 weeks of administration of rhGH (1.4 mg/d, or one quarter of the dose utilized in the first study); rhIGF-1 (5 mg twice daily); rhGH with rhIGF-1; or placebo in 60 patients with AIDS-associated wasting. In part because of a large dropout rate, these workers noted only transient increases in body weight and lean body mass and decline in fat mass in the groups receiving rhGH or rhIGF-1 alone; in the group receiving rhGH plus rhIGF-1 these changes persisted for 12 weeks. Increase in muscle strength and improvement in quality of life also were transient. In neither study was there any alteration in immune function or exacerbation of AIDS. Waters et al concluded that rhGH and rhIGF-1 at the doses employed in their study were not useful in the treatment of the wasting of AIDS.

Waters D, et al. Recombinant human growth hormone, insulin-like growth factor 1, and combination therapy in AIDS-associated wasting: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1996;125:865-872.

Schambelan M, et. al. Recombinant human growth hormone treatment and HIV-associated wasting. *Ann Intern Med* 1996;125:873-882.

Editor's comment: *Although rhGH, rhIGF-1, or a combination of the 2 agents can increase lean body mass and decrease body fat content in adults with HIV- and AIDS-associated wasting (at least transiently), beneficial effects on the course of the disease or the quality of life were not observed in either of the 2 studies. Despite such data, the Food and Drug Administration has approved a 12-week course of rhGH therapy for patients with HIV-associated wasting. The estimated cost per patient is $12,000.*

Allen W. Root, MD

Adult Height in Growth Hormone Deficiency (GHD) Children Treated With Biosynthetic GH

This study consists of the largest number of patients (121) treated over the longest period with a constant dose (0.3 mg/kg/wk) of rhGH reported to date. One hundred six patients completed the study and attained their adult height. The chronologic age at initiation of therapy was 11.3 ± 2.1 years for males and 10.1 ± 2.8 years for females. The etiology of GHD was 102 idiopathic versus 19 organic. Eighty-four of the 121 developed puberty spontaneously. The total duration of GH treatment (with or without native pituitary GH) was approximately 7.5 ± 3.2 years. The Bayley-Pinneau predicted adult height was 163.2 ± 7.4 cm for males and 150.0 ± 7.0 cm for females. The adult statistics are recorded in the table to the right.

Adult height was dependent on height (positively) and age (negatively) at the start of these protocols, duration of treatment on protocol, growth rate during first year, and sex.

The authors postulate that the significant increase in adult height over pretreatment predicted adult height may be due to larger doses of uninterrupted GH treatment than that used in previous studies. The authors also found, in contrast to previous studies, that spontaneous puberty or female sex did not adversely affect the adult height SDS, which improved significantly during puberty from

Adult Height in Subjects Treated With rhGH

Outcome Variable	Males (n=72)	Females (n=49)
Adult height (cm)[a]	171.6 ± 8.2	158.5 ±7.1
Total height gained (cm)[b]	46.6 ± 11.8	41.6 ± 16.5
Adult height SD score	-0.7 ± 1.3	-0.7 ± 1.1
Adult height SD score minus midparental target height SD score[c]	-0.6 ± 1.2(n=66)	-0.4 ± 1.2 (n=45)
Age at onset of puberty[a]	14.0 ± 1.9	12.6 ± 2.2
Height at start of puberty (cm)[a]	146.7 ± 10.5	139.1 ± 11.8
Height SD score at start of puberty	-1.9 ± 1.2	-1.9 ± 1.5
Adult height minus predicted adult height at start of treatment (cm)[b]	8.5 ± 8.1	8.5 ± 7.1

Values are the mean ± SD.
[a] By *t* test, $P<0.0003$, males versus females.
[b] By paired *t* test, each sex, $P< 0.0001$ (different from 0).
[c] By paired *t* test: females, $P=0.02$; males, $P<0.0001$ (different from 0)

-1.9 ± 1.3 to -0.7 ± 1.2, and maximum stimulated GH and more frequent GH injections were significant predictors of first-year growth rates. They were not predictive of adult height or adult height SDS.

Blethen SL, et al. *J Clin Endocrinol Metab* 1997:82:418-420.

Editor's comment: *This is the most definitive study done to date to answer the questions posed when the study was first begun. Short GHD children attain normal heights when the diagnosis of GHD is made early and adequate GH therapy to produce catch-up growth is initiated early in life.*

Robert M. Blizzard, MD

Low-Dose Recombinant Human Growth Hormone Increases Body Weight and Lean Body Mass in Patients With Short Bowel Syndrome

A randomized, double-blind, placebo-controlled, crossover study using low-dose (0.17 mg/kg/wk) recombinant hGH in individuals with short bowel syndrome (SBS) due to Crohn's disease is reported. Ten adults with surgically created SBS for more than 1 year participated in the study. All had normal 24-hour growth hormone profiles. Their mean small intestinal length was 1.3 m; 6 of the 10 had an ileojejunostomy. All required oral or parenteral fluids. One was maintained on parenteral nutrition. Lean body fat, bone mineral content, and bone mineral density were measured by dual-energy X-ray absorptiometry (DEXA). Fecal samples were collected on a metabolic ward and pooled in 4-day batches.

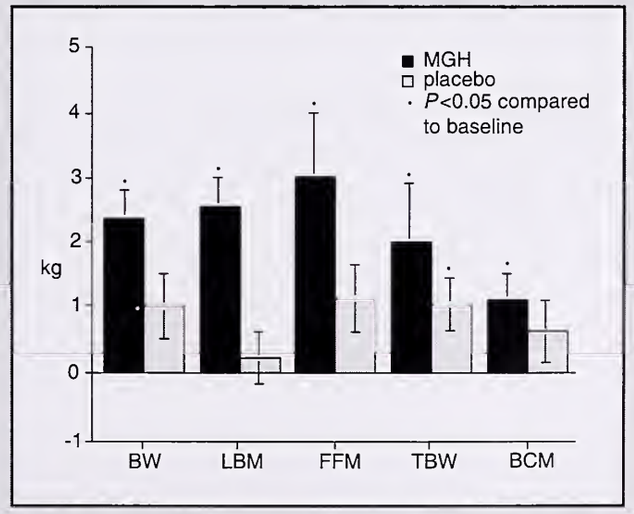
During treatment with rhGH, body weight increased by 2.3 ± 0.8 kg (P=0.005) (Figure 1). Lean body mass increased 5.6 ± 1.9% (P=0.005) while body fat did not change significantly. There were small but significant changes in bone mineral content but no significant changes were seen in total body bone mineral density. Mean daily energy intake from food was 3,500 kcal. Urinary nitrogen excretion did not change during this study, but nitrogen balance was significantly improved, 4.8 ± 2.9 g/d versus 2.3 ± 2.9 g/d (P=0.011). The authors suggest that these studies demonstrate that short-term, low-dose GH therapy for as little as 8 weeks can increase body weight, lean body mass, total body water, and bone mineral content without clinical signs of edema or altered glucose metabolism. Thus, this may be a useful adjunct to nutritional support for patients with SBS.

Ellegard L, et al. *Ann Surg* 1997;225:1:88-96.

Editor's comment: *These interesting studies suggest ways in which the anabolic effects of GH may be useful in individuals with SBS secondary to Crohn's disease. Recently, growing adolescents with inactive Crohn's disease (*Digestive Diseases and Sciences *1996;41:1754-1759) were reported to have increased energy expenditure as compared to both healthy growing adolescents and non-growing subjects with inactive Crohn's disease. Until we understand more about the pathophysiology of Crohn's disease, suggesting alternatives to increased nutrient intake as a means of improving growth in these individuals may not be possible. However, the studies by Ellegard et al suggest that individuals with Crohn's disease and SBS may benefit significantly from the anabolic effects of rhGH. Byrne et al (*Annals of Surgery *1995;222:243-255) studied rhGH in addition to a high-carbohydrate, low-fat diet with added glutamine in 17 adults with SBS and demonstrated significant improvement in absorption of protein and decrease in stool output. Although the studies performed by Byrne et al and those reported above by Ellegard and colleagues were performed in adults, the potential implications for children with congenital or acquired SBS are apparent. Randomized, multicenter trials are currently in progress using both pediatric and adult populations to evaluate this new therapeutic regimen. The estimated potential reduction in health-care costs associated with this treatment should be an incentive for industry support of these studies. There is reason to be optimistic that this could be an additional beneficial use of rhGH.*

William L. Clarke, MD

Figure 1



Changes in body composition during 8 weeks of treatment with low-dose recombinant human growth hormone (rhGH) and placebo in 10 patients with short bowel syndrome. BW = body weight; LBM = lean body mass; FFM = fat-free mass; TBW = total body water; BCM = body cell mass.

From Ellegard L, et al. Low-dose recombinant human growth hormone increases body weight and lean body mass in patients with short bowel syndrome (SBS). Lippincott-Raven Publishers, NY. *Ann Surg* 1997;225(1):92.

Trisomy 21: A Possible Molecular Basis

Trisomy 21 accounts for the vast majority of Down syndrome. It is the most common trisomy of newborns and the leading known cause of mental retardation. Trisomy 21 has long been known to result from failure of the 2 homologous chromosome 21s to segregate (nondisjunction) during meiosis, especially during maternal meiosis I. Recently, evidence has emerged that the genetic recombination that normally occurs between homologous chromosomes during meiosis is altered in trisomy 21. Indeed, reduced recombination confined primarily to the proximal region of chromosome 21q was found in cases due to meiosis I errors. Now, abnormal recombination also has been found in cases due to meiosis II errors. In the meiosis II errors, recombination is increased, and accounts for about 20% of trisomy 21.

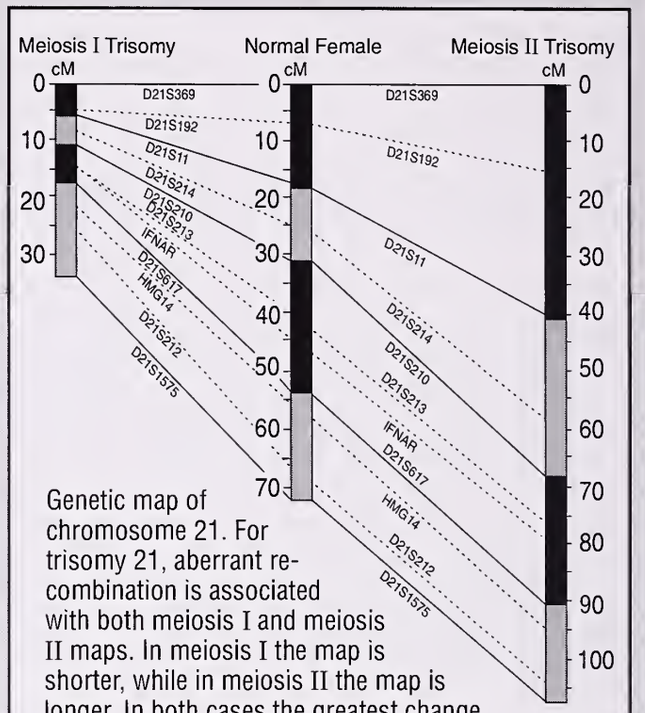
Lamb et al studied 133 trisomy 21 cases of maternal meiosis II errors using a panel of chromosome 21 DNA markers that allowed them to examine genetic recombination as well as the parental and meiotic origin of the extra chromosome. They found increased recombination restricted mainly to the proximal q arm of the chromosome. Importantly, they detected no difference in the extent of recombination in chromosomes derived from older versus younger women.

The observations prompted speculation from these authors, as well as from Orr-Weaver in an invited editorial, about how decreased and increased recombination might contribute to chromosome segregation errors in meiosis I and meiosis II, respectively. The deviations from "normal" are depicted in the genetic map of chromosome 21, shown in Figure 1, in which the length of chromosome segments corresponds to the extent of recombination. It is suggested that physical attachments that exist between homologous chromosomes during recombination (so-called chiasmata, or sites of crossing over) and between sister chromatids are important for normal chromosome segregation. In the former instance, it is proposed that chiasmata that form near the end of the chromosome are less effective at promoting proper segregation than those formed proximally. Perhaps distal chiasmata are less stable than proximal ones. If so, a reduction in proximal chiasmata, which would be associated with the observed reduced recombination in this region, would predispose to missegregation at meiosis I.

To explain how increased recombination events in the proximal 21q might promote meiosis II errors, the possibility of chromosome entanglement is raised. In this scenario, some of the excessive proximal chiasmata are not resolved during meiosis I. This results in failure of the chromosome 21 homologues to segregate. If the homologues remain entangled after the first segregation, then their chromatids may not segregate properly during meiosis II. This implies that disturbances of meiosis I can adversely affect segregation at meiosis II — a new concept.

Since neither of these explanations addresses why trisomy 21 occurs more frequently in older mothers, a 2-step process is proposed. In the first step, "susceptible" meiotic chromosome configurations are established prenatally in all female fetuses. In most instances, such configurations are resolved by normal meiotic processes. However, with increasing age, these processes become less effective and unresolved susceptible configurations result in nondisjunction and trisomy. Meiotic-specific proteins, such as spindle or microtubular motor proteins, that degrade with time are mentioned as candidates to explain the age-dependency of the meiotic errors.

Figure 1



Genetic map of chromosome 21. For trisomy 21, aberrant recombination is associated with both meiosis I and meiosis II maps. In meiosis I the map is shorter, while in meiosis II the map is longer. In both cases the greatest change in length occurs proximal to 21q.

Adapted with premission from Orr-Weaver T. *Nature Genet* 1996;14:374-376.

Lamb NE, et al. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. *Nature Genet* 1996;14:400-405.

Orr-Weaver T. Meiotic nondisjunction does the two-step. *Nature Genet* 1996;14:374-376. Editorial.

Editor's comments: *History has tended to keep the disciplines of cytogenetics and molecular genetics apart in many institutions. The work described above importantly demonstrates the value of integrating the 2 to address questions that have been around for decades. The results may not explain precisely why trisomy 21 occurs, but they provide hypotheses to test and molecular contexts in which to consider alternative possibilities.*

William A. Horton, MD

Dwarf Mice and the Aging Process

Brown-Borg et al have reported that Ames dwarf mice (*df/df*) live longer than their normal siblings of the same strain given the same environmental conditions. The authors followed 28 normal and 34 Ames dwarf mice born during July and August 1992 from the same litters. Both types of mice were maintained in a conventional environment and fed the same unrestricted lab chow and tap water. The male dwarf mice lived 350 days longer than normal male mice and the female dwarf mice lived 470 days longer than the normal female mice. Mean age at death for normal males is 723 ± 54 days; for normal females 718 ± 45 days; for dwarf males 1,076 ± 56 days; and dwarf females 1,206 ± 32 days.

Ames dwarf mice are characteristically normal size at birth but severely growth retarded after birth and are approximately one-third normal size as adults. They have primary pituitary deficiency, including absence or extreme reduction in GH, prolactin, and thyroid-stimulating hormone. The GH/IGF-1 axis is markedly depressed, and the mice exhibit reduced immune function.

The mechanisms suggested for longevity in these genetically dwarf mice are related to low GH and IGF-1 levels; low thyroid-stimulating hormone and thyroid hormone levels and hypogonadism; reduced metabolic rate, possibly due to reduced body size and underlying endocrine defects; reduced caloric intake; and failure of sexual maturation.

Brown-Borg HM, et al. *Nature* 1996;384:33. Letter.

Editor's comment: *Aging is a complex process influenced by genetic and environmental forces. Genes and hormones, especially GH, IGF-1, and sex hormones, appear to play a role in longevity. It is well known that individuals with elevated GH levels resulting in acromegaly and pituitary gigantism have a shorter life span. The observation that female dwarf mice lived longer than male mice also suggests that female hormones play some role in the aging process. These animal models are important for studying the effects of hormones on growth, aging, and the aging process, and almost surely will improve our understanding of the aging process in both rodents and humans.*

Judith G. Hall, MD

Mechanisms and Treatment of Growth Retardation in Children With Liver Transplants

Sarna et al report on their experience with 18 months of rhGH treatment, beginning at least 18 months after liver transplant, in 8 children (5 boys, 3 girls). A total of 41 children have had liver transplants with a 70.2% graft survival after 1 year. The inclusion criteria for the study were: age > 2 years; liver transplant at least 18 months previously; height SD score (SDS) < -2.0 or growth velocity SDS <0 for chronologic age and sex; bone age < 14 years in boys and ≤ 13 years in girls; and no serious complications due to transplantation. The patients were treated with 1.0 IU/kg/wk (approximately 0.3 mg) rhGH. They were measured at 2 weeks, 6 weeks, and 3 months, and at 3-month intervals thereafter using a Harpenden stadiometer. Height SDS was calculated.

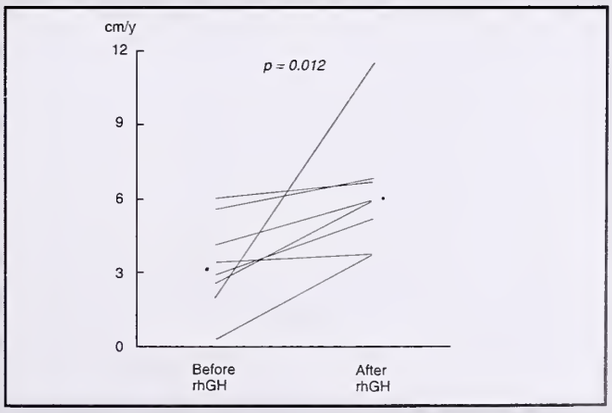
These 8 children received their liver transplants for a variety of causes, including hepatoblastoma (3), biliary atresia (4), and α_1-antitrypsin deficiency (1). The median growth rate increased from 3.2 to 6.0 cm/y (P=0.012) (Figure 1). The median height SDS increased from -3.9 to -3.0 (P=0.036) during treatment. The individual growth responses did not correlate wtih baseline age, time elapsed after transplant, nocturnal GH secretion, serum IGF-1, or IGFBP-3. No rejection episodes were documented during treatment.

Sarna S, et al. *Transplant Proc* 1997;29:447-448

Editor's comment : *The patients in this study had a significantly accelerated linear velocity despite receiving low doses of glucocorticoids (amount not specified). The authors state that traditional predictors of response to rhGH such as low GH secretory status and young age were not shown to be predictors of a good response in the current study. The study itself included too few subjects to be able to characterize individuals who might benefit the most from GH therapy. This editor hopes that a larger, multicenter, multinational study could be performed so that such variables can be clearly identified. Since GH has some effects on the immune system, it is important to continue to monitor liver function tests closely during its administration. Although the authors conclude that "growth response is variable and difficult to predict," it is not unreasonable to expect that such information might be forthcoming from future studies.*

William L. Clarke, MD

Figure 1



Growth response (cm/y) in 8 children with liver transplants during the first 18 months of recombinant human growth hormone (rhGH) treatment. The black circle represents the median.

From Sarna S, et al. Mechanisms and treatment of growth retardation in children with liver transplants. *Transplant Proc* 1997;29:448. With permission Elsevier Science Inc.

Post Program Self-Assessment/CME Verification

Instructions: The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. GHRP-6 was named this because:
   a) It was the 6th GH-releasing peptide developed.
   b) It was 6 times the potency of GH-releasing hormone.
   c) It was a hexapeptide.
   d) It was active both in vivo and in vitro.
   e) It was developed in 1986.
2. Which of the following substances cause the release of GH from the pituitary?
   a) MK-0677; a spiropiperdine
   b) Prolactin-releasing hormone
   c) Hexarelin
   d) GH-releasing hormone
3. GHRP and the nonpeptidyl GH secretagogues:
   a) Use the same receptor.
   b) Both stimulate the secretion of GH in vitro and in a variety of species in vivo.
   c) Both are active when given IV, IM, SC, intranasally, and orally.
4. The following are true statements:
   a) The GH-releasing activity of GHSs is inhibited by somatostatin.
   b) The GH-releasing activity of combined GHRH and GHRP are synergistic.
   c) GHSs stimulate greater secretion of GH than does GHRH.
   d) Although a positive GH secretory response to GHS implies the presence of somatotrophs and their exposure to GHRH, an absent or blinded response does not identify the site of error in GH secretion.
5. Threonine is substituted for methionine at codon 918 in the RET tyrosine kinase in which disease phenotype?
   a) FMTC
   b) MEN 2A
   c) MEN 2B
   d) MEN 2A & 2B
6. One possible explanation for the divergence of MEN 2A & 2B phenotype during RET activation may be:
   a) Dimerization and autophosphorylation of the receptor tyrosine kinase during ligand binding.
   b) Dimerization and activation of tyrosine kinases in the absence of ligand which is characteristic of MEN 2A.
   c) The fusion of the RET tyrosine kinase domain to sequences from different cellular proteins.
   d) RET mutations along cell lines exposed to in vitro irradiation.
7. Which statement is false?
   a) BCR-ABL fusion proteins have severalfold elevation of tyrosine activity over that in the normal c-ABL kinase.
   b) Tyrosine kinase activation of the BCR-ABL protein correlates with disease phenotype.
   c) There is an inverse correlation between the activity level of the BCR-ABL protein and the rate of disease progression.
   d) BCR-ABL has transforming ability in many in vitro assays of tumorigenicity.
8. By what mechanism can tyrosine kinase become constitutively activated?
   a) Autocrine production of growth factor
   b) Mutation
   c) Concomitant production of a growth factor and its receptor
   d) All of the above
9. MEN 2A may be characterized by all but:
   a) Average age of onset in the second decade
   b) Pheochromocytomas
   c) Parathyroid disease
   d) Developmental abnormalities, such as characteristic facies, marfanoid habitus, and skeletal abnormalities

Answer Key: 1. c 2. a,c,d 3. a,b,c 4. a,b,c,d 5. c 6. b 7. c 8. d 9. d

Disclosure: As mandated by the ACCME, all faculty participating in continuing medical education programs sponsored by the University of Virginia School of Medicine are expected to disclose to the program audience any real or apparent conflicts of interest related to the content of their presentation.

Drs. Bercu, Kolibaba, Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Diamond is President, Genentech Endowment for Growth Disorders, an independent foundation funded by Genentech to provide growth hormone to financially needy children. Dr. Druker reports grants from Ciba-Geigy, NIH, and The Leukemia Society for laboratory tests to investigate tyrosine kinase inhibitors. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development which functions independently of Genentech, Inc.

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Molecular Genetics of Human Chondrodysplasias

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INTRODUCTION

The human chondrodysplasias are a diverse and genetically heterogeneous group of disorders of skeletal development.[1] They especially affect linear bone growth and are due to mutations that disrupt endochondral ossification in the skeletal growth plate. The target events include the proliferation and differentiation of chondrocytes and the coincident production of cartilage that serves as a template for bone formation and gives rise to the articular surfaces of joints.

Defining the molecular genetics of the chondrodysplasias has been difficult because they are individually rare and because families large enough to be informative for linkage studies are uncommon. Nevertheless, a number of "chondrodysplasia" loci have now been identified.[2] This article provides a brief overview of recent progress in this field.

COL2A1

A mutation of the type II collagen gene (*COL2A1*) was first reported in 1989 in affected members of a family with spondyloepiphyseal dysplasia (SED) congenita. Since then, the number of *COL2A1* mutations in patients with the SED class of chondrodysplasias has risen to well over 30.[3] Examples of such mutations are shown in Figure 1, which also depicts the structure of collagen alpha chains, the product of the collagen genes. All mutations have been heterozygous, involving only 1 of the 2 *COL2A1* alleles. They map to the triple helical domain of the molecule. Most involve the substitution of glycine residues that are considered critical to proper assembly of the triple helix.

The clinical picture has ranged from achondrogenesis type II and hypochondrogenesis at the severe end of the spectrum to very mild SED with precocious osteoarthritis, often referred to as late-onset SED, at the other end. Clinical phenotypes of intermediate severity include SED congenita and Kniest dysplasia. *COL2A1* mutations also have been found in Stickler dysplasia, in which the skeletal phenotype is typically dominated by degenerative arthritis rather than short stature as well as eye and inner ear abnormalities.

Several interesting observations have been made regarding the clinical features that result from particular *COL2A1* mutations, so-called genotype/phenotype correlations. For instance, there is a tendency for mutations that alter amino acids residing toward the C terminus of the collagen triple helix to produce more severe phenotypes compared with those altering amino acids toward the N terminus of the helix. This phenotypic gradient is similar to that observed for mutations of *COL1A1* and *COL1A2* in osteogenesis imperfecta.

Letter From the Editor

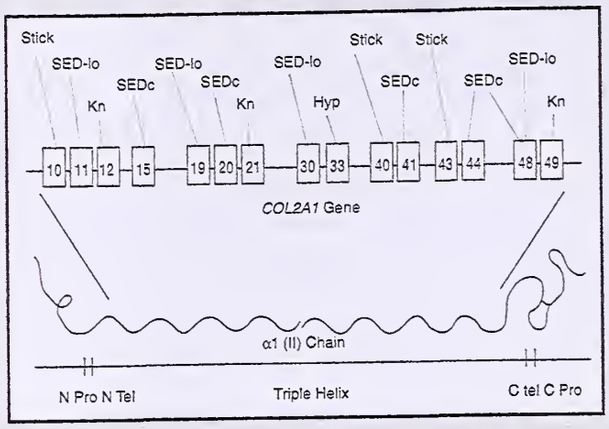
Our readers may be interested in knowing that the glossary concerning genetic terms, which was published in *GGH* (1997;13[2]), has now been put on a web page, with appropriate credit given to Drs. Judith Hall and William Horton, who are responsible for constructing this wonderful glossary. You may wish to make notations concerning this glossary, which can be brought up on the computer at your immediate command, and hang them in your clinic or other appropriate places. The URL is: http://www.kumc.edu/gec/gloss.html. Ms. Debra Stultz, Program Manager, Genetics Education Center, University of Kansas Medical Center, Kansas City, Kansas, is responsible for placing the glossary on the site, and we thank her for taking the initiative to do this.

Robert M. Blizzard, MD

In This Issue

This educational activity is supported in part by an unrestricted educational grant from Genentech, Inc.

Figure 1
***COL2A1* MUTATIONS**



Schematic of *COL2A1* mutations. Type II collagen chain (α1[II] chain) near bottom with domains listed underneath. A portion of the *COL2A1* gene encoding the triple helical domain with exons numbered is drawn above. Representative chondrodysplasias with SED or SED-like clinical phenotypes are depicted above with lines indicating to which exons the mutations map. Abbreviations: C pro=carboxy propeptide; C tel=carboxy telopeptide; Hyp=hypochondrogenesis; Kn=Kniest dysplasia; N Pro=amino propeptide; N tel=amino telopeptide; SED-lo=SED-late onset; SEDc=SED congenita; Stick=Stickler dysplasia.

From Horton WA. Molecular genetic basis of the human chondrodysplasias. *Endocrinol Metab Clin North Am* 1996; 25:683-697.

However, as with osteogenesis imperfecta, there are many exceptions.

Three *COL2A1* mutations have been reported in patients with Kniest dysplasia that are predicted to interfere with splicing of mRNA transcripts. The consequence would be partial or complete deletion of exons that encode parts of the type II collagen triple helix. Such deletions are expected to disrupt assembly of collagen molecules; however, the reasons why they should produce the clinical features of Kniest dysplasia are not understood.

The mutations discussed until now are thought to act through a dominant negative mechanism at the molecular level. Since collagen molecules are comprised of 3 alpha chains, there are theoretically 8 possibilities for how the products of 2 *COL2A1* alleles can combine. If 1 allele is mutant, then half of the alpha chains will be mutant and 7 of the 8 possible combinations will contain at least 1 mutant chain. In general, the fate of collagen molecules containing mutant chains is not well understood. The most often proposed possibilities include premature degradation, which would reduce the abundance of type II collagen in cartilage matrix, and incorporation of the mutant molecules into cartilage collagen fibrils, adversely affecting their functions. Most likely, some combination of these and perhaps other events occurs.

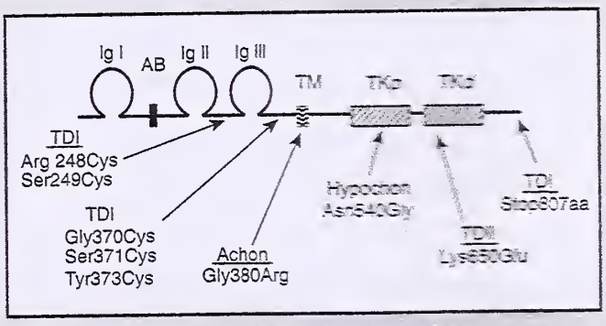
The product of the *COL2A1* gene also is the alpha 3 chain of type XI collagen. Accordingly, phenotypic consequences of *COL2A1* mutations may be due in part to defects in the synthesis and functions of type XI collagen.

In contrast to the dominant negative action of most *COL2A1* mutations, those associated with Stickler dysplasia are believed to act by so-called haploinsufficiency. For example, 7 of the heterozygous mutations reported to date create premature translation stop signals, most often because the reading frame of the transcript is shifted so that an out-of-frame stop codon is encountered downstream. The result is that collagen chains synthesized from such transcripts are truncated. Most importantly, they lack the noncollagenous C propeptide, which is necessary for incorporation into triple helical molecules.

Somatic mosaicism has been found for *COL2A1* mutations. In one instance, the proband presented with the features of Kniest dysplasia. Somatic mosaicism was detected in the patient's mother, who exhibited mild skeletal abnormalities consistent with Stickler dysplasia. It also was found in the father of another patient with Kniest dysplasia. His features were compatible with late-onset SED.

As noted above, heterozygous mutations of *COL2A1* are thought to act through dominant negative or haploinsufficiency mechanisms to introduce mutant collagen molecules in the extracellular matrix of cartilage in the former instance and/or to reduce the abundance of the protein in cartilage matrix in both circumstances. Precisely how either phenomenon disrupts skeletogenesis is not well understood. However, since cartilage serves as a template for endochondral ossification and since type II collagen is the principal structural protein of cartilage, it follows that cartilage containing abnormal or deficient type II collagen would not function properly as a template.

Figure 2



Schematic of *FGFR3* depicting structure of the receptor and sites of common mutations. Abbreviations: Ig I, II, and III = immunoglobulin domains I, II, and III; AB = acid box; TM = transmembrane domain; TK*p* = proximal tyrosine kinase domain; TK*d* = distal tyrosine kinase domain; TDI and TDII = thanatophoric dysplasia I and II; Achon = achondroplasia; Hypochon = hypochondroplasia; aa = translated amino acid.

Adapted from Horton WA. Molecular genetic basis of the human chondrodysplasias. *Endocrinol Metab Clin North Am* 1996;25:683-697.

COL11A2

Mutations of *COL11A2*, the gene encoding the alpha 2 chain of type XI collagen, have been described in 2 families with a syndrome that closely resembles Stickler dysplasia.[4] Affected family members presented with mild SED, precocious osteoarthritis, and sensorineural hearing loss, all of which are characteristic of Stickler dysplasia; however, they lacked the eye abnormalities typical of the condition. The explanation for the latter exception lies in the fact that the alpha 2 chain of type XI collagen does not contribute to type XI collagen molecules in the eye as it does in other tissues.

After genetic linkage to this locus was established in 1 large family, subsequent analysis revealed a splice site mutation in 1 allele. In the second family, the Stickler-like syndrome presented as an autosomal recessive trait with 3 affected sibs and unaffected parents. The affected sibs had more extensive epiphyseal and hearing abnormalities than usual for Stickler dysplasia. It is of note that the sibs were fourth cousins and also that osteoarthritis was common on the paternal side of the family. The affected sibs were homozygous for a glycine mutation while both parents were heterozygous. Type XI collagen is a minor constituent of cartilage matrix, especially in the growth plate. It is thought to help regulate the size of cartilage collagen fibrils. How mutations disturb this or other functions is not understood.

COL10A1

A number of heterozygous mutations have been reported in the gene for type X collagen (*COL10A1*).[5] They are all associated with the clinical features of the relatively mild Schmid-type metaphyseal chondrodysplasia. The mutations map to the region of the gene that encodes the C propeptide, and are of the types predicted to disrupt association of chains, the first step of helix formation. The effect is that half of the type X collagen chains cannot be incorporated into molecules, reducing the number of molecules by half. How a partial deficiency of type X collagen disturbs bone growth is not known. The protein is found only in the hypertrophic zone of the skeletal growth plate, where it is thought to facilitate endochondral ossification.

FGFR3

Achondroplasia is by far the most common chondrodysplasia in humans. As a result, the research for the achondroplasia gene was more extensive than that for any other chondrodysplasia. In early 1994, the locus was mapped to a region of about 2.5 Mb of DNA at the tip of the short arm of chromosome 4 (4p16.3). Ironically, it mapped very near to another elusive disease gene locus, the Huntington disease locus. This was relevant because a number of genes in this region had been characterized as candidates for the Huntington gene, and one of these, the fibroblast growth factor receptor 3 gene (*FGFR3*), turned out to be the achondroplasia gene.[6]

Most remarkable about the achondroplasia mutations was that virtually every patient with typical achondroplasia had mutations at the same site: codon 380, which caused the normal glycine residue to be replaced by an arginine residue (Gly380Arg) (Figure 2). The Gly380Arg mutation has now been detected in several hundred patients.

The observations in achondroplasia prompted the search for *FGFR3* mutations in 2 disorders thought to be related: thanatophoric dysplasia (TD) and hypochondroplasia. These exhibit greater and lesser degrees of severity relative to achondroplasia, respectively. Mutations were quickly discovered in TD.[7] They clustered mainly to 4 locations in the *FGFR3* gene, as shown in Figure 2. The 2 forms of TD that had been implicated from skeletal X-ray studies, TDI and TDII, segregated genetically in that all TDII cases and no TDI cases had the Lys650Glu mutation. An interesting set of TD mutations removes the normal translation stop signal. The consequence of this mutation is that the FGFR3 protein would be 141 amino acids longer than normal if translation continues to the next in-frame stop codon.

Heterozygous *FGFR3* mutations also were detected in hypochondroplasia.[8] As with the other *FGFR3* mutations, they cluster, in this case, to amino acid residue 540, where asparagine is replaced by lysine (Asn540Lys). On clinical grounds, 1 patient was thought to be a compound heterozygote for both achondroplasia and hypochondroplasia. The parents carried the respective diagnoses and the severity was intermediate between heterozygous and homozygous achondroplasia, as would be predicted for such a compound. Both the hypochondroplasia (Asn540Lys) and achondroplasia (Gly380Arg) alleles were found at this patient's *FGFR3* loci.

Thus, with few exceptions, human achondroplasia class mutations map to a limited number of codons within different regions of the *FGFR3* gene that correspond to domains of the mature protein. In other words, achondroplasia mutations map to the transmembrane domain, hypochondroplasia mutations to the proximal tyrosine kinase domain, TDII mutations map to the distal tyrosine kinase domain, and so on.

There has been much speculation about how *FGFR3* mutations interfere with skeletal development.

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Table 1
Summary of Human Chondrodysplasia Mutations

Locus	Chromosomal Location	Gene Product Function	Clinical Phenotype	Inheritance Pattern	Proposed Effect of Mutation
COL2A1	12q13.11	Extracellular matrix protein	Achondrogenesis type II Hypochondrogenesis SED congenita Kniest dysplasia Last-onset SED Stickler dysplasia	AD AD AD AD AD AD	Dominant negative Dominant negative Dominant negative Dominant negative Dominant negative Haploinsufficiency
COL9A2	1p33-p32.3	Extracellular matrix protein	MED	AD	Dominant negative
COL10A1	6q21-q22	Extracellular matrix protein hypertrophic cartilage	Schmid-type metaphyseal chondrodysplasia	AD	Haploinsufficiency
COL11A2	6p21.3	Extracellular matrix protein	Stickler-like dysplasia	AD AR	Dominant negative Loss of function
COMP	19p12-13.1	Extracellular matrix protein	Pseudoachondroplasia MED Fairbanks MED Ribbing	AD AD AD	Dominant negative Dominant negative Dominant negative
FGFR3	4p16.3	Tyrosine kinase transmembrane receptor for FGFs	Thanatophoric dysplasia Achondroplasia Hypochondroplasia	AD AD AD	Gain of function Gain of function Gain of function
PTHrPR	3p21-p22	G protein transmembrane receptor for PTH and PTHrP	Jansen-type metaphyseal chondrodysplasia	AD	Gain of function
DTDST	5q31-q34	Transmembrane sulfate transporter	Diastrophic dysplasia Atelosteogenesis type II Achondrogenesis type IB	AR AR AR	Loss of function Loss of function Loss of function
ARSE	Xp22.3	Arylsulfatase enzyme	Chondrodysplasia punctata	XLR	Loss of function
SOX9	17q24.1-q25.1	Transcription factor	Campomelic dysplasia	AD	Haploinsufficiency

Adapted from Horton WA. Molecular genetics of the human chondrodysplasias. *Eur J Hum Genet* 1995;3:357-373.

The bulk of the evidence supports the idea that they activate receptor signaling in the absence of ligand (constitutive activation) and that the extent of activation varies according to the mutation and correlates with the severity of the clinical phenotype. For example, hypochondroplasia mutations activate the signaling pathway to a small extent while TD mutations activate it much more. The downstream elements of the *FGFR3* pathway are not well defined. Presumably, they influence the proliferation and differentiation of growth plate chondrocytes.

Given that virtually all TD mutations and most achondroplasia mutations arise de novo, the mutation rate of the *FGFR3* locus must be very high. Most remarkable, however, is the mutation rate at the codons most often involved, especially the 380 codon, for which the mutation rate is much higher than other highly mutable human genes. There is no satisfactory explanation for this extremely high mutation rate.

COMP

As its name implies, pseudoachondroplasia resembles achondroplasia. However, its clinical features differ substantially, and it is a genetically distinct autosomal dominant chondrodysplasia. It was linked to chromosome 19p12-13.1 about 3 years ago. The gene for cartilage oligomeric matrix protein (*COMP*) was mapped to this region in late 1994, and mutations in *COMP* were found in pseudoachondroplasia soon afterwards.[9] *COMP* is a member of the thrombospondin family of proteins. It is found in the extracellular matrix of cartilage and, to a lesser extent, other connective tissues, including ligament and tendon. Its function in these tissues is not well defined.

COMP mutations have been identified in a number of patients with pseudoachondroplasia. They are heterozygous and map mainly to regions of the gene encoding calmodulin repeats. These repeats are a distinctive element of the molecule; they are thought to bind calcium, which is necessary for the correct folding of the molecule.

At the same time that pesudoachondroplasia was mapped to chromosome 19p12-13.1, linkage to this site also was established for the Fairbanks type of multiple epiphyseal dysplasia (MED) in 1 large family. The locus was termed EDM1. Subsequent analysis of genomic DNA from MED patients revealed a mutation in the *COMP* gene, establishing that pseudoachondroplasia and some forms of MED are allelic disorders and presumably share common pathogenetic features. *COMP* mutations have subsequently been detected in the Ribbing form of MED.

COMP mutations are believed to act through a dominant negative mechanism. Indeed, the molecule normally exists in extracellular matrix as a pentomer. If one considers the ways in which the products of a mutant and a normal allele can combine into pentomeric molecules, only 1 of 32 possible combinations contains 5 normal *COMP* monomers; 31 contain at least 1 mutant monomer. It has been proposed that this dominant negative effect disrupts *COMP* synthesis and secretion, leading to accumulation of abnormally folded molecules inside cells and/or a deficiency of *COMP* outside cells of relevant tissues.

COL9A2

MED was genetically mapped in a large family to chromosome 1 in 1994. The locus was named EDM2 to distinguish it from the EDM1 locus on chromosome 19, which is now known to be the *COMP* locus. When the *COL9A2* locus was located in the chromosome 1 region of interest, it was considered a strong candidate gene for this family. A heterozygous mutation was subsequently detected in several members.[10] Type IX collagen is a quantitatively minor cartilage collagen thought to participate in the regulation of collagen fibril assembly in cartilage matrix. The mutation is assumed to behave in a dominant negative fashion. It should be noted that genetic linkage to both the *COMP* (EDM1) and *COL9A2* (EDM2) loci has been excluded in 1 family with the clinical picture of MED.

CME CERTIFICATION

The *GGH* Editorial Board is pleased to announce Category 1 credit for *GROWTH, Genetics, & Hormones* from the University of Virginia School of Medicine. This enduring material has been planned and produced in accordance with the ACCME Essentials.

Overview: This enduring material is designed to provide physicians and other health professionals with current research and clinical information essential to providing quality patient care to children with growth problems and genetic disorders.

Target Audience: This enduring material is designed for pediatricians, pediatric endocrinologists, pediatric geneticists, and family medicine physicians interested in pediatric growth, genetics, and endocrine issues.

Method of Physician Participation: Physicians can study each issue of *GROWTH, Genetics, & Hormones*, respond to the post-test self-evaluation questions, and request CME credit for each issue. The estimated length of time to complete this enduring material is 1 hour.

Learning Objectives: Through participation in this enduring materials series, the participant will have the opportunity to:

1. Apply current research and advances to the management of patient care for optimum clinical outcomes.
2. Utilize current research and clinical care issues to initiate discussions with colleagues with a focus toward increased awareness of current issues and controversies.
3. Conceptualize areas for future research in the field of growth and genetics.

DTDST

The autosomal recessive chondrodysplasia, diastrophic dysplasia (DTD), was mapped to chromosome 5q in 1990, and a gene encoding a sulfate transporter was identified as harboring DTD mutations 4 year later.[11] The gene was designated *DTDST* for diastrophic dysplasia sulfate transporter. Several mutations have now been described for DTD. In addition, mutations of *DTDST* also have been detected in 2 other autosomal recessive chondrodysplasias: the more severe atelosteogenesis type II and the much more severe achondrogenesis type IB.[12] Interestingly, some of the same mutant alleles have been found in DTD and the other disorders. In fact, it appears that different combinations of mutant alleles determine the severity of the clinical phenotype.

The nature of *DTDST* mutations as well as the recessive inheritance of the disorders suggest that the phenotypes result from different degrees of loss of function of the sulfate transporter protein. Compared with other tissues, cartilage is very rich in proteoglycans, such as aggrecan, whose glycosaminoglycan side chains are heavily sulfated, ie, chondroitin sulfate and keratan sulfate. There is evidence that cartilage glycosaminoglycans are poorly sulfated in *DTDST* disorders.

PTHrPR

The Jansen type of metaphyseal chondrodysplasia is a distinctive autosomal dominant chondrodysplasia that shares many phenotypic features with the acquired vitamin D deficiency disease, rickets. Since many of the manifestations of rickets reflect overactivity of parathyroid hormone (PTH), investigators searched for abnormalities in PTH and its receptor, which also serves as a receptor for another hormone called PTH-related protein, or PTHrP. While the hormone studies were unremarkable, the PTHrP receptor analysis revealed a mutation in a patient with Jansen-type metaphyseal chondrodysplasia.

The heterozygous mutation causes a histidine-to-arginine substitution in the PTHrP receptor protein.[13] This histidine is highly conserved among members of the G protein-coupled transmembrane receptor family to which the PTHrP receptor belongs. Substantial evidence suggests that this mutation as well as one other are activating mutations, ie, the receptor is activated in a ligand-independent fashion.

Recent studies indicate that PTHrP signaling serves as a "brake" on terminal differentiation of chondrocytes in the growth plate. Activating mutations of the PTHrP receptor would be expected to enhance this braking effect, presumably slowing bone growth.

ARSE

Chondrodysplasia punctata (CDP) refers to a heterogeneous group of skeletal dysplasias in which abnormal calcium deposits form in cartilage tissues to pro-

duce stippled epiphyses on X-ray films. An X-linked recessive form of CDP has been mapped to the short arm of the X chromosome (Xp22.3), near the boundary of the pseudoautosomal region of the X chromosome. When this region was searched for genes, 3 adjacent genes were found that encoded previously unrecognized sulfatase enzymes. Because of predicted structural similarities to arylsulfatases A, B, and C (ARSA, ARSB, and ARSC), the novel sulfatase genes were named *ARSD, ARSE,* and *ARSF.* Mutations in the *ARSE* gene were found in several boys with X-linked recessive CDP. Some of the patients exhibited severely reduced *ARSE* enzyme activity in a cell culture assay.

Defects in sulfate metabolism might be expected to be expressed in cartilage, given the high degree of sulfation of matrix constituents (above). However, the mechanism by which they cause calcium in deposits in cartilage is not evident.

SOX9

Campomelic dysplasia is a chondrodysplasia associated with sex reversal, ranging from minor abnormalities of external genitalia to complete sex reversal. Previous reports of chromosomal rearrangements in campomelic dysplasia with sex reversal localized the gene(s) responsible to chromosome 17q24.1-q25.1. Recently, the candidate region was placed near the *SOX9* locus. *SOX9* is a member of a family of transcription factor genes structurally related to the *SRY* (sex-determining region Y) gene, which encodes a factor necessary for testicular development in mammals. The mature protein contains a high mobility group (HMG) domain and a proline- and glutamine-rich domain believed to confer DNA-binding and transcription-activating properties, respectively.

Mutations of *SOX9* have now been reported in a number of patients with campomelic dysplasia, including those with and without sex reversal.[14] Several of the mutations predict that translation would be stopped prematurely, truncating the transcription factor so that the activation domain is missing. The vast majority are heterozygous, supporting autosomal dominant inheritance. Evidence of somatic mosaicism for a mutant allele in maternal lymphocytes was found in one instance. The mother had no overt signs of campomelic dysplasia and had given birth to a normal XX girl previously.

Given the inactivating nature predicted for the mutations, they most likely act through haploinsufficiency. It has been suggested that transcription regulation functions of *SOX9* are dose-dependent. *SOX9* transcripts have been demonstrated in growth plate chondrocytes of growing bones; however, the functions are unknown.

CONCLUSIONS

Several conclusions can be drawn from these observations, as summarized in Table 1. First, the concept of chondrodysplasia families, which was originally based on finding qualitative similarities in clinical phenotypes, has been confirmed at the molecular level. Mutations of *COL2A1* and *FGFR3* in the SED and achondroplasia classes of disorders demonstrate this well. The characteristics of the mutations, however, differ substantially.

Mutations of *COL2A1* are dispersed throughout the gene, and mutations in genes whose products interact functionally with type II collagen, ie, *COL11A2,* cause similar phenotypes. Genetic heterogeneity is the rule. The evidence to date suggests that the "generic" SED phenotype results from dysfunction of cartilage collagen fibrils during bone growth and maintenance of articular cartilage. Since such fibrils, are comprised of types II, IX, and XI collagen molecules, mutations in any of the contributing genes could potentially give rise to an SED or SED-like phenotype. Subtle differences in the functions that constituent molecules have and in the consequences of specific mutations account for particular SED phenotypes. The MED phenotype seems to show similar genetic heterogeneity, with mutations having been found at the *COMP* and *COL9A2* loci and linkage to another locus implicated in other MED families.

In contrast, mutations of *FGFR3* display the opposite phenomenon of genetic homogeneity; they cluster to only a few codons, and there is a remarkable fidelity with regard to particular mutations giving rise to particular clinical entities. This implies a very high degree of specificity with regard to the biologic sequelae of signaling through mutant *FGFR3* receptors. Subtle differences in the degree of constitutive activation probably determine the particular clinical phenotypes. A similar phenomenon may be found in *PTHrPR* mutations as more are identified.

The extremely high rate of spontaneous mutation of certain *FGFR3* codons is of considerable interest. Given the high degree of sequence conservation in the vicinity of the mutations in mammals, one would expect to find comparable mutations in other species, especially the mouse, where such phenotypic variants have been carefully monitored. However, there appears to be no such mutant mouse, nor is there a satisfactory explanation for the discrepancy.

Allelism within the chondrodysplasias deserves comment. In several instances, suspected allelism has been confirmed, as in the disorders associated

In Future Issues

Insulin, the IGF System, and IDDM
Cheryl Deal, MD

How Safe and Effective Is Human Growth Hormone at Pharmacologic Dosing?
Arnold Slyper, MD

The Therapeutic Use of Growth Hormone in Turner Syndrome and Other Non-GH–Deficient States
Ron Rosenfeld, MD

with *COL2A1* and *FGFR3* mutations. In some cases, allelism came as a surprise, as for pseudoachondroplasia and MED on the one hand and DTD, atelosteogenesis type II, and achondrogenesis type IB on the other. On the contrary, the Schmid and Jansen types of metaphyseal chondrodysplasia, which share at least enough phenotypic similarity to be classified together, turn out to result from mutations of quite different genes: one encoding an extracellular matrix protein, the other a transmembrane receptor. These findings indicate that clinical phenotypes are not always good predictors of genotypes.

Finally, the number of chondrodysplasia loci seems to be shrinking. A decade ago, when little was known about the loci, it was predicted that the number would be large, given the complexity of skeletal development. The recent advances, however, have demonstrated that mutations at the *COL2A1* and *FGFR3* loci alone account for a great majority of patients with chondrodysplasias. Thus, despite the complexity, the number of genes critical for skeletal growth for which redundancy cannot compensate for mutations is not as large as originally suspected.

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Abstracts From the Literature

Growth Hormone in Combination With Anabolic Steroids in Patients With Turner Syndrome: Effect on Bone Maturation and Final Height

In an uncontrolled trial of the efficacy of rhGH and androgens on growth of girls with Turner syndrome (TS), the authors report the final heights of 20 patients treated for 4.0 to 7.7 years (see Figure 1). Initially, all children received rhGH alone for 12 to 30 months (either 12 or 18 IU/m²/wk, or approximately 4 to 6 mg/m²/wk); an androgen in the form of oxandrolone (0.0625 mg/kg/d) or testosterone (5 mg IM every 2 weeks) was then introduced; after 12 to 24 months the testosterone-treated children were changed to oxandrolone until the end of the therapeutic trial. Estrogen was introduced after a bone age ≥ 12.5 years was achieved. (This ranged from 13.0 to 19.6 years; mean age, 16.3 ± 1.7 years.) A progestin was added after approximately 15 months. At final height (growth rate < 2.0 cm/y), the children were 15 to 23 years of age. The mean projected adult height (PAH; derived from Austrian data on untreated patients with TS) was 143.7 ± 4.0 cm (range, 137.5 to 151.0 cm); the mean achieved final height was 152.9 cm (range, 145.0 to 158.9 cm); the mean increment above PAH was 9.3 ± 4.9 cm (range, 1.4 to 21.4 cm). Relative to target height (target height minus achieved height), mean final height was -9.6 ± 5.3 cm lower, but the range of final heights relative to target heights was -2.4 ± 18.7 cm; one patient reached and one exceeded target height. Treatment before or after 11.5 years did not affect outcome. The authors concluded that rhGH and androgen can result in a substantial increase in adult stature in girls with TS.

Haeusler G, et al. *Acta Paediatr* 1996;85:1408-1414.

Editor's comment: *The data indicate that the combination of rhGH and androgen can increase final height in girls with TS. The use of rhGH in girls with TS has been approved by the Food and Drug Administration (FDA). The results of the present study, however, were achieved with postponement of estrogen therapy until an average age of 16 years. There is no discussion by the authors of the impact of this delay in initiation of puberty on the psychosocial well-being of these patients. It is this writer's practice to introduce estrogens into the treatment program of girls with TS at 13 to 14 years of age, depending on individual circumstances. Other investigators[1,2] have not recorded comparable achievements in final heights in girls with TS treated with rhGH alone.[1-4] Until long-term data on the quality of life (educational achievement, psychosocial well- being, career attainment) of patients with TS are available, the efficacy of rhGH therapy on this important point remains conjectural.*

Allen W. Root, MD

1. Hindmarsh PC, et al. *Lancet* 1996:348:13-16.
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3. Van den Broeck J, et al. *J Pediatr* 1995;127:729-735.
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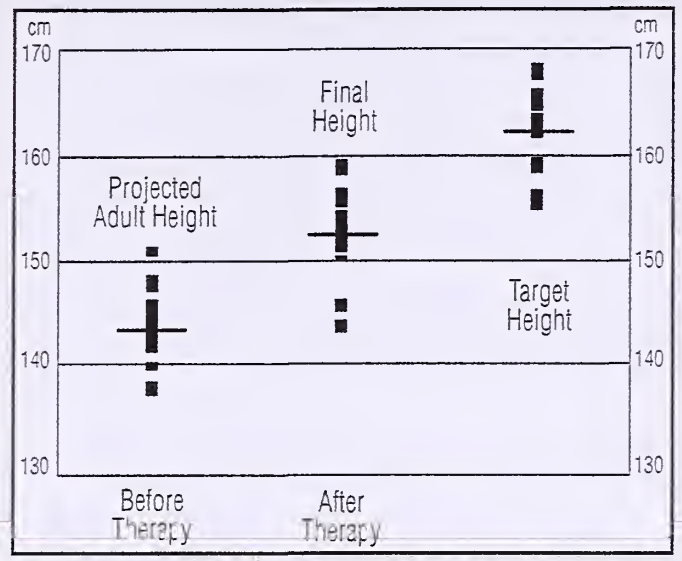
2nd Editor's comment: *Members of the Editorial Board do not always agree, but such disagreement can lead to constructive contemplation. My personal view is that this article considers at least 4 issues: (1) Does rhGH alone significantly increase the ultimate height of patients with TS? (2) Does oxandrolone enhance the action of rhGH in patients with TS? (3) Does the timing of estrogen administration alter the ultimate height of TS patients if given early during the teen years? (4) If estrogen is given in the late*

Figure 1



Auxologic data of 20 patients with TS at start of therapy and after 4.0 to 7.7 years of therapy.

From Haeusler G, et al. Final height after GH and anabolic steroids in Turner syndrome. *Acta Paediatr* 1996;85:1411.

teens instead of in the early teens, is this delay psychologically handicapping to TS patients?

The FDA recently has approved the use of rhGH for the long-term treatment of short-stature associated with TS. Thus, it has been recognized as an agent that increases the average ultimate height of TS patients. Whether this improvement is significant is in the eyes of the beholders, which is not necessarily the same in the eyes of TS patients and non-TS patients. The data prompting approval by the FDA are presented in part in an article by Rosenfeld et al, published in the Journal of Pediatrics. *These authors studied 70 TS children, verified by karyotype. A prospective study was used. In an initial phase lasting 12 to 24 months, subjects were randomized to either (1) observation alone, (2) oxandrolone, (3) GH, or (4) GH plus oxandrolone. After completion of the first phase, subjects on GH alone continued to receive only GH. All other subjects were treated with GH plus oxandrolone. Data from this trial were compared with growth characteristics of 25 American historical control subjects with TS—matched for age, height, parental target height, and karyotype—who never received GH or androgens.*

Of the 70 subjects enrolled, 60 completed the clinical trial. The 17 subjects receiving GH alone all completed the trial and reached a mean height of 150.4 ± 5.5 cm. This was 8.4 ± 4.5 cm taller than their mean PAH at enrollment (95% confidence interval, 6.3 to 10.6 cm). The 43 subjects receiving GH plus oxandrolone attained a mean height of 152.1 ± 5.9 cm; this was 10.3 ± 4.7 cm taller than their mean PAH. The historical controls had a mean adult height of 144.2 cm ± 6.0 cm, precisely matching their original PAH of 144.2 ± 6.1 cm.

The authors concluded that GH, either alone or with oxandrolone, is capable of both stimulating short-term growth and augmenting adult height in TS girls. With early diagnosis and initiation of treatment, an adult height > 150 cm is a reasonable goal for most girls with TS.

In respect to the second question: Having been involved with the combined use of rhGH and oxandrolone in GH deficiency and in TS over many years, I have a strong impression that not only is growth accelerated with the combination but also, possibly, ultimate height, providing oxandrolone is not used before the bone age is 9 years and the dose does not exceed 0.1 mg/kg/d. The data of Rosenfeld et al and Haeusler et al support this impression.

In respect to the third and fourth questions: I believe whether estrogen is administered early or late in the teen years should be a personal decision of the TS patient. Unequivocally there is a trade-off: Early sexual development hastens epiphyseal fusion and decreases ultimate height. Some girls prefer sexual development to greater ultimate height and some do not.

Finally, the cost of rhGH, which is expensive, must be taken into account. This cost cannot be ignored and must be factored into the decision making of the patient, the family, and others.

Again, my opinion is that of only one member of the Editorial Board. The use of rhGH will be considered further in a future article to be published in GGH, entitled, "How Safe and Effective Is hGH at Pharmacologic Dosing?" In the meantime, Letters to the Editors are most welcome.

Robert M. Blizzard, MD

SHOX, Short Stature and Turner Syndrome

Turner syndrome is one of the most common and most widely studied forms of short stature. It classically results from absence of an X or a Y chromosome, leaving the patient with a 45,XO karyotype. However, a subset of patients have only deletions of the X or Y. Many of these deletions map to the pseudoautosomal region (PAR1) at the tips of the chromosome short arms. From analysis of short stature in such patients, a 700-kb interval of PAR1 has recently been implicated to contain the gene(s) involved in short stature in Turner syndrome.

With this as a starting point, Rao et al did further deletion mapping to narrow the interval associated with short stature to 170 kb. This critical region was deleted in 36/36 patients with short stature and rearrangement of Xp22 or Yp11.3. From extensive analysis of cosmids covering this interval, they identified 3 exons of a novel homeobox-containing gene, which they named *SHOX*. *SHOX* appears to be alternatively spliced to produce transcripts (*SHOXa* and *SHOXb*) that yield proteins 292 and 225 amino acids in length, respectively. Expression studies revealed that *SHOXa* is widely expressed, whereas *SHOXb* is more restricted, and is predominantly expressed in bone marrow fibroblasts.

These results strongly suggest that haploinsufficiency for SHOX proteins causes short stature in Turner syndrome.

If so, isolated mutations of *SHOX* should produce short stature in the absence of other Turner syndrome features. Accordingly, screening of 91 unrelated male and female patients with idiopathic short stature yielded 1 patient with a heterozygous missense mutation predicted to truncate the protein such that the functions of both the 225 and 195 amino acid proteins would be potentially disturbed The mutation segregated with short stature in the family. In an accompanying News and Views report, Zinn cautioned that evidence for haploinsufficiency of *SHOX* causing short stature in Turner syndrome is not definitive even though it is substantial.

SHOXa has a very similar sequence to the mouse gene called *OG-12a*, which encodes a protein of unknown function. *OG-12a* does not map to the mouse X chromosome. The authors speculate that if *OG-12a* is the mouse *SHOX* homologue, its non-X chromosome location may explain why the XO-deficient "Turner syndrome" mouse does not exhibit short stature.

Rao E, et al. *Nat Genet* 1997;16:54-63.
Zinn AR. *Nat Genet* 1997;16:3-4.

Editor's comment: *This article would appear to settle the argument over whether short stature in Turner syndrome is caused by loss of 1 or loss of several genes that influence stature, although as Zinn points out, the evidence is not definitive since there could still be other growth-influencing genes whose expression is affected by chromosomal rearrangements. Of interest is what* SHOX *gene products do in the growing bone. Unfortunately,* SHOX *expression was not studied in cartilage, most notably in the growth plate. Nevertheless, the high level of expression of* SHOXb *in bone marrow fibroblasts, which are physically in close proximity to the growth plate, is intriguing. It will be interesting to see if expression of the putative* SHOX *transcription factors affects expression of growth factors that might diffuse into and influence growth plate function.*

William A. Horton, MD

2nd Editor's comment: *Dr. Jay Ellison of the University of California–San Francisco wrote in a Letter to the Editor: "Our group has cloned the gene termed* "SHOX" *independently, which we named* "PHOG" *(pseudoautosomal homeobox-containing osteogenic gene). We have demonstrated significantly higher levels of expression in certain bone-derived cells. Its deletion in patients with short stature, the predicted altered dosage in 45,X individuals, and the nature of the encoded protein and its expression pattern make* PHOG *an attractive candidate for involvement in the short stature of Turner syndrome. We also have found that the mouse homologue of* PHOG *is autosomal, which may help to explain the lack of growth abnormality in mice with monosomy X."*

Readers need to be aware of the dual nomenclature. This gene, no matter what it is called, will be an important consideration in exploring the various causes of short stature. A number of significant questions remain, including what is the growth mechanism that is controlled by SHOX/PHOG*?*

Robert M. Blizzard, MD

3rd Editor's comment: *Normal growth is undoubtedly regulated by many genes, including genes others than* SHOX *on the Y chromosome, as discussed by Zinn. In the paper by Rao et al, the authors have defined a homeobox gene (*SHOX*) in PAR1 of the sex chromosomes (Xp22 and Yp11.3) that seems to influence growth, since it is absent in short patients who lack this portion of one sex chromosome and a mutation leading to truncation of its gene product has been identified in all but 1 of 91 individuals with intrinsic short stature. The genetic mechanisms through which* SHOX *regulates growth have yet to be determined. It will be of interest to determine if polymorphic variations of this gene influence the range of heights characteristic of a population.*

Allen W. Root, MD

Human Chromosomes in Mice

The introduction of foreign DNA into the mouse genome, ie, transgenesis, is an extremely powerful tool for elucidating molecular and cellular disturbances that contribute to human diseases. The limiting factor in this technology has been the size of foreign DNA that can be incorporated into the host genome. In the early days of transgenic mice, only small fragments of genes could be transferred. This has improved steadily with successes in introducing entire genes, including local regulatory sequences, and more recently in transferring substantial amounts of DNA in the form of yeast artificial chromosomes (YACs). Interest has developed in generating human artificial chromosomes (HACs) that could be introduced like YACs. However, a report from a Japanese group headed by Isao Ishida indicates that this may not be necessary since human chromosomes themselves can function as vectors in transgenic mice.

The group successfully introduced human chromosomes or chromosome fragments from fibroblasts into mouse embryonic stem (ES) cells via microcell-mediated chromosome transfer. The ES cells were transferred to preimplantation mouse embryos, where they populated a wide variety of developing tissues to generate chimeric mice. The foreign chromosomes, which potentially contained thousands of human genes, survived repeated cell divisions as well as differentiation of cells into many cell types. Several of the human genes were shown to be expressed under tissue-specific regulation in adult chimeric mice. Most notable were immunoglobulin genes, which were found to undergo rearrangement of V, J, and D

segments to generate functional immunoglobulins that were detected in the mouse serum for more than a year. Finally, the group demonstrated transmission of a human chromosome 2 fragment to offspring through both the male and female germlines.

Tomizuka K, et al. *Nat Genet* 1997;16:133-143.
Rastan S. *Nat Genet* 1997;16:113-114.

Editor's comment: *There are several implications from this work. The most remarkable is that human chromosomal elements can interact with mouse mitotic, meiotic, and transcriptional machinery to ensure mendelian transmission and appropriate expression of genes carried by the human chromosome vectors. The authors refer to such mice as transchromosomic mice. This technology will allow investigations not previously possible, such as studies of how distant regulatory elements influence gene expression or how functionally related, contiguous genes, such as* globin *or* hox *genes, are regulated. Phenomena that involve interactions between neighboring genes, such as imprinting, might be studied in such mice, as might aneuploid states such as trisomy 21. Finally, it is often debated whether science drives technology or vice versa. This seems to be an example of the latter, although there is an abundance of scientific questions to be answered by this new technology.*

William A. Horton, MD

"Master Gene" for Bone Formation

The discovery of the transcription factor MyoD as a master gene for muscle development several years ago prompted a search for similar genes for other tissues. Four papers in the May 30, 1997, issue of *Cell* describe a gene of comparable importance to osteoblast differentiation and bone growth. The findings were nicely reviewed by Rodan and Harada.

The story begins with efforts by Komori and colleagues to disrupt T-cell function by knocking out a gene encoding the transcription factor *Cbfa1* (core-binding factor). The factor is a member of the *runt*-domain gene family of developmentally important transcription factors originally described in *Drosophila*. It was known to bind to promoters of genes expressed in T cells. When the knockout occurred, however, the most dramatic features had more to do with skeletal development than with T-cell function. The null mice died shortly after birth. They were slightly smaller than their normal littermates and had shorter limbs, but were normally proportioned. Most remarkable was a complete absence of bone. Both membranous and endochondral bones of the cranium and endochondral bones elsewhere were absent. In their place was partially calcified cartilage. No osteoblasts were detected and osteoclasts were reduced in number. There was no obvious problem with hematopoiesis.

Cbfa1 null mice generated independently by Otto et al showed essentially the same findings. They also demonstrated that *Cbfa1* is normally expressed in areas destined to become bone in mouse embryos. They noticed that mice heterozygous for the knockout displayed ossification defects of the clavicles and membranous bones of the skull similar to that seen in humans with the autosomal dominant disorder, cleidocranial dysostosis (CCD). A mouse with a similar phenotype resulting from a radiation-induced deletion had been studied by the Olsen group[4] as a model for CCD. It was quickly determined that *Cbfa1* mapped to the region of interest and that it was disrupted by the radiation-induced deletion.

The next step was to test CCD patients for *CBFA1* mutations. Mundlos et al studied 39 patients and found large and small deletions, insertions, and missense mutations that inactivated *CBFA1*. The mutations were heterozygous in all of the patients.

As these studies were being done, the Karsenty lab (Ducy et al) had started from a different perspective: identifying osteoblast-specific transcription factors (OSFs) that bind to the osteocalcin promoter. Osteocalcin is a bone-specific protein. They had cloned such a factor, which they named Osf2. Its expression was restricted to the early stages of osteoblast differentiation, and it was shown to bind to several genes expressed by osteoblasts. They also showed that forced expression of Osf2 in nondifferentiated cells induced expression of osteoblast-specific genes and that the presence of antisense mRNA downregulated expression of these genes in osteoblastic cells.

As it turned out, Osf2 and *Cbfa1 (CBFA1)* are the same. Thus, this transcription factor seems to be required for differentiation of osteoblasts and for normal skeletal development and growth. Haploinsufficiency for *CBFA1* causes CCD.

Ducy P. et al. *Cell* 1997;89:747-754.
Komori T, et al. *Cell* 1997;89:755-764.
Mundlos S. et al. *Cell* 1997;89:773-779.
Otto F. et al. *Cell* 1997;89:765-771.
Rodan G, Harada S. *Cell* 1997;89:677-680.

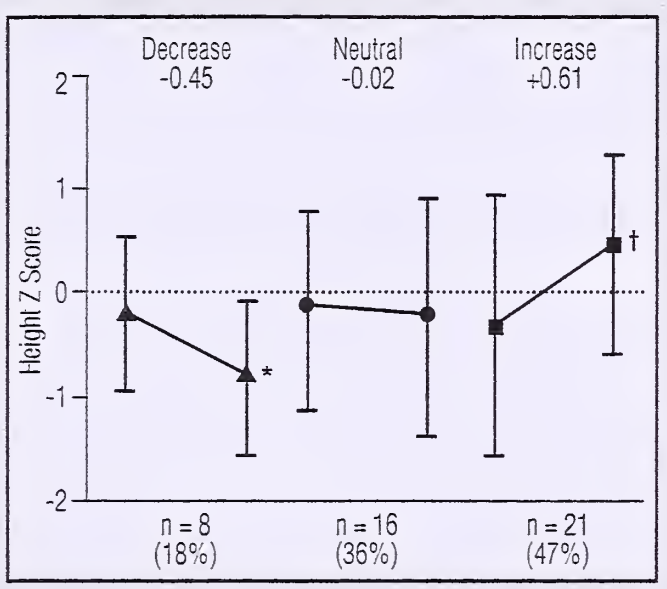
Editor's comment: *This is a fascinating story with important implications for understanding bone growth. The fact that the skeletal structures formed and "grew" to close to normal length in the newborn mice null for* Cbfa1 *suggests that conversion of the cartilage template to bone is not as essential for bone lengthening as many would guess. The photomicrographs suggest that new cartilage is deposited at the ends of lengthening "bone" regardless of the fate of hypertrophic cartilage in the center of the structure. This implies that the signals that drive chondrocyte proliferation and at least early hypertrophy in the growth plate are not derived from subchondral bone, ie, from osteoblasts or other cell types that normally reside in bone marrow. However, such signals may be necessary to complete the terminal differentiation process that occurs in the growth plate, since this seemed to be lacking in the null mice. These mice should be valued for studying the events that occur at the interface between cartilage and bone in a growing bone. One wonders what the status of* Cbfa1 *is in the shark skeleton.*

William A. Horton, MD

Widespread Growth Retardation and Variable Growth Recovery in Foster Children in the First Year After Initial Placement

The authors hypothesized that the actual prevalence of preplacement growth failure may be greater than that defined by a single cutoff percentile, eg, the 5th percentile, at placement. The objective was to determine the growth pattern of 45 children, 1.5 to 6.0 years of age, in the first year of foster care placement. Height, weight, weight for height, and annual growth velocity Z scores at 1 year after placement, as compared with baseline values, were used as outcome measures. All children received comprehensive medical care.

Figure 1



Change in height Z score for each of 3 types of responders: those with a decrease (loss in height Z score of > 0.25), those with an increase (gain in height Z score of > 0.25), and those between these extremes (neutral). Asterisk indicates that median Z score is significantly different from baseline (P=.008); dagger, P<.001.

From Wyatt DT, et al. Widespread growth retardation and variable growth recovery in foster children in the first year after initial placement. *Arch Pediatr Adolesc Med* 1997;151:815. Copyright 1997. American Medical Association.

The changes in height Z scores are plotted in Figure 1. Forty-seven percent experienced catch-up growth (gain in height Z = + 0.61) that equaled that seen in the first year of GH therapy in children with classic GH deficiency. The authors interpreted these data as reflecting prior growth retardation.

The authors drew the following conclusions: (1) Growth retardation is widespread in children placed in foster care, with almost half showing marked catch-up growth after placement; (2) initial height is not a good predictor of future growth; (3) the use of cutoff percentiles (only 5 of the 45 were <5th percentile by actual measurement at baseline) will miss the great majority of children who will show catch-up growth; and (4) the response of any given child is variable and cannot be accurately predicted by any baseline auxologic feature. In the authors' experience, 1 in 5 children in foster care experiences a significant loss in height Z score after placement, which may indicate ongoing medical or nutritional problems, unmet psychosocial needs, or failure of the foster care family. The causes of growth failure preplacement and subsequent catch-up growth are unclear, but they may be related mainly to psychosocial factors that are corrected for in some children with foster care placement.

Wyatt DT, et al. *Arch Pediatr Adolesc Med* 1997;151:813-816.

Editor's comment: *These data and the conclusions reached are well documented. The authors are to be congratulated for an important clinical investigative study. The use of Z scores is essential to analyses of the data and the conclusions. The authors state that future analyses of data in this study will examine the relationships between growth and other medical, developmental, and psychologic diagnoses; the amount and type of healthcare services received; changes in health and mental status; and the quality of the foster home environment. The editors of* GGH *encourage the authors to expedite these analyses and reports. The study of this group is exceedingly important.*

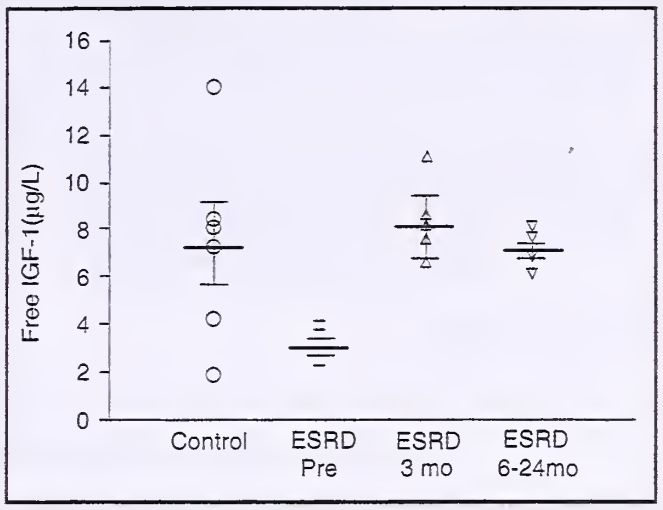
Robert M. Blizzard, MD

Growth Hormone Treatment in Growth-Retarded Children With End-Stage Renal Failure: Effect on Free/Dissociable IGF-1 Levels

One of the causes for growth retardation in children with end-stage renal disease (ESRD) is thought to be an abnormality in the biologic effects of GH. Despite high serum levels of hGH in ESRD and usually normal values of insulin-like growth factor 1 (IGF-1), somatomedin biologic activity is low. This has been attributed to binding of IGF-1 by an excess of IGF-binding proteins (IGFBPs), leading to decreased free IGF-1 concentrations.

Bereket et al tested this hypothesis by measuring free IGF-1 by direct immunoradiometric assay (IRMA) in 5 children with ESRD. In 2, free IGF-1 also was measured after centrifugal ultrafiltration of serum. Free IGF-1 concentrations were one third to one half those measured by direct IRMA, suggesting that the IRMA measured both free IGF-1 and that fraction that was easily dissociable from IGFBPs. In basal specimens, the mean free/dissociable IGF-1 levels were lower in ESRD patients than in body mass index-, age-, and pubertal status-matched control subjects (3.0 ± 0.3 µg/L vs 7.3 ± 2.1 µg/L; 1.24 ± 0.05% vs 2.12 ± 0.7%, respectively). The mean free/dissociable IGF-1 peaked at 8.5 ± 1.0 µg/L after 3 months of treatment with rhGH, declining to 6.9 ± 1.4 µg/L between 6 to 24 months of therapy (Figure 1). Growth rate and total IGF-1 values also rose during rhGH administration. Thus, the increase in growth rate during rhGH administration was associated with a rise in free/dissociable IGF-1 levels.

Figure 1



Serum free IGF-1 concentrations in control children and children with chronic renal failure before, at 3 months, and at 6 through 24 months of GH treatment. The horizontal lines and vertical bars indicate the mean and SEM in each group.

From Bereket A, et al. Growth hormone treatment in growth retarded children with end stage renal failure: effect on free/dissociable IGF-1 levels. *J Pediatr Endocrinol Metab* 1997;10:200. Freund Publishing House Ltd.

Bereket A, et al. *J Pediatr Endocrinol Metab* 1997;10:197-202.

Editor's comment: *These data support the concept that the growth-promoting effects of rhGH in children with ESRD is related to an increase in free IGF-1 concentrations. In this paper, the authors did not report a relationship between the basal or incremental growth rate and the concentration or incremental increase in free/dissociable IGF-1 values. Additional studies will be helpful in clarifying fully the mechanisms by which rhGH increases growth in ESRD.*

Allen W. Root, MD

Pancreatic Agenesis Attributable to a Single Nucleotide Deletion in the Human *IPF1* Gene Coding Sequence

IPF1 is a homeodomain protein critical for development of the pancreas in mice and is a key factor for the regulation of the insulin gene in the beta cells. Disruption of this gene in transgenic mice produces failure of pancreatic development. In this report, a single nucleotide deletion within codon 63 in a patient with pancreatic agenesis apparently does the same. The patient was homozygous for the point deletion and both parents were heterozygous, in contrast to the normal allele structure in 184 individuals. The cytosine deletion was in codon 63. A frameshift beginning at the C-terminal border of the transactivation domain of *IPF1* was consistent in all cells. The data indicated that a truncated protein lacking the homeodomain (and nuclear localization signal) is produced from the mutation. If the parallel between humans and affected mice holds, the pancreatic buds do form, but they undergo only limited ductal outgrowth and branching, with a blockage of both pancreatic endocrine and exocrine differentiation. Although there was no clear history of consanguinity, the studies strongly suggest that the abnormal alleles are likely to have been derived from a single common ancestor.

In addition to pancreatic agenesis, 3 cases of severe pancreatic hypoplasia and 1 case of complete absence of the islets have been reported. The authors are tempted to speculate that the phenotypes of pancreatic hypoplasia and selected agenesis of the islets might represent a spectrum of less severe mutations that may impair but not abolish *IPF1* functions. Alternatively, these disorders may be a consequence of mutations and other factors that are essential for full development of the pancreas. Most intriguingly, the authors postulate that abnormal *IPF1* function also may be a candidate factor in the development of insulin-dependent diabetes mellitus.

Stoffers DA, et al. *Nat Genet* 1997;15:1-30. Letter.

Editor's comment: *An intriguingly rare condition is probably explained by these investigators. Recently, a white female infant was diagnosed with pancreatic agenesis shortly after birth, and with pancreatic exocrine insufficiency at 18 days of age. Neonatal diabetes mellitus was the working diagnosis initially. Ultrasound examination demonstrated pancreatic agenesis. Normal development has continued until 5 years of age with replacement of insulin and pancreatic enzymes. A strong family history of noninsulin-dependent diabetes mellitus existed and supports the possibility of partial affectation.*

Pancreatic agenesis needs to be considered in the differential diagnosis of neonatal diabetes and also with the observation of malabsorption in the newborn period. A similar study of the IPF1 *gene coding sequence might be revealing in the Johanson-Blizzard syndrome, which is characterized by pancreatic insufficiency in addition to other anomalies such as congenital deafness, poor formation of teeth, corneal atresia, and urogenital anomalies.*

With time it becomes more and more apparent that one mis-substitution of an amino acid at a critical place on a gene can totally change the life of the host far beyond what we ever could have believed 10 years ago.

Robert M. Blizzard, MD

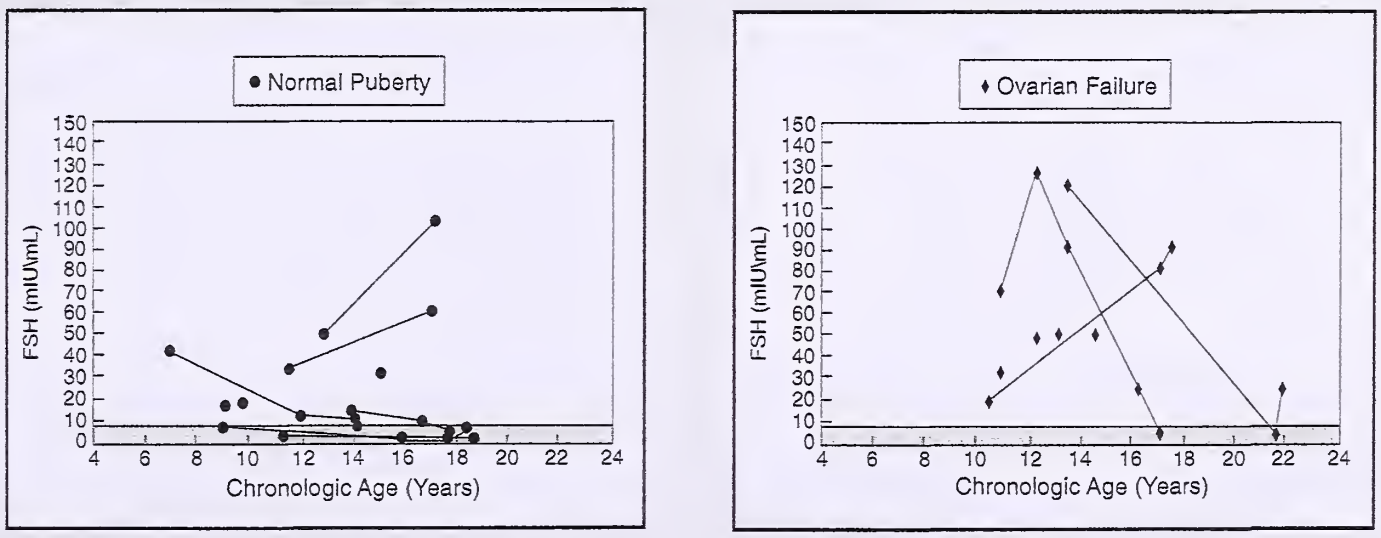
Gonadal Function After Bone Marrow Transplantation for Acute Leukemia During Childhood

Bone marrow transplantation (BMT) is a major advancement in the treatment of childhood leukemia and in other disorders, as many children are surviving for long periods. Consequently, Sarafoglou et al examined the impact of BMT on gonadal function following high-dose chemotherapy and hyperfractionated total body irradiation (radiation given 3 times daily for several days) in 33 surviving children treated for acute leukemia. All patients were prepubertal and less than 12 years at the time of BMT. The median age at last examination for boys was 14 years (10.4 to 17.1 years) and 16.9 years (9.5 to 21.9 years) for girls.

Of the 17 boys, 14 (82%) had spontaneous puberty, 13 (76%) had age-appropriate plasma concentrations of testosterone; 2 (11%) remained clinically and hormonally prepubertal; and 1 (6%) had overt Leydig cell failure requiring androgen replacement therapy, although this individual also received testicular irradiation. Thirty-six percent of pubertal boys had increased levels of luteinizing hormone (LH), reflecting evidence of Leydig cell damage; and 64% had increased levels of follicle-stimulating hormone (FSH), reflecting germ cell damage. Pubertal boys with increased LH were significantly younger at BMT (5.4 ± 0.8 years vs 7.8 ± 0.8 years).

Of 16 girls, 9 (56%) had spontaneous puberty with onset of menarche at a median age of 13 years (9.5 to 15.8 years). Six of these 9 girls (67%) had increased LH and in-

Figure 1



Plasma concentrations of FSH in girls after BMT with normal puberty/menarche *(left panel)* and in girls with ovarian failure *(right panel). Solid lines* connect serial determinations in the same patient. *Shaded area* represents the range for the normal population (follicular phase of the menstrual cycle). BMT, bone marrow transplantation; FSH, follicle-stimulating hormone.

From Sarafoglou K, et al. Gonadal function after bone marrow transplantation for acute leukemia during childhood. *J Pediatr* 1997;130:214.

creased FSH. Seven of 16 girls (44%) required hormone replacement because of clinical and biochemical evidence of ovarian failure (Figure 1). One of the 16 (6%) recovered ovarian function after 5.5 years. Girls with ovarian failure were significantly older at BMT compared with those with spontaneous puberty/menarche (8.6 ± 2.3 years vs 6.1 ± 1.8 years).

The authors concluded that most prepubertal boys undergoing BMT with chemotherapy and hyperfractionated total body irradiation can expect to have normal puberty. For prepubertal girls, approximately 50% retained sufficient ovarian function to enter puberty and menstruate regularly. Increased age at transplantation was associated with decreased ovarian function in girls.

Sarafoglou K, et al. *J Pediatr* 1997;130:210-216.

Editor's comment: *Two factors seem to be associated with gonadal dysfunction in these youngsters: (1) age at BMT, and (2) pubertal status. Many children who are younger at BMT seem to retain or recover gonadal function and are able to enter spontaneous puberty. The study documented that spontaneous puberty occurred in 82% of boys and 56% of girls, which is quite encouraging for males but less reassuring for females, almost half of whom fail to attain spontaneous puberty. Therefore, the ovaries appear to be more prone to damage with irradiation and high-dose chemotherapy. Not surprisingly, there is an association with direct testicular irradiation and gonadal failure. With improvement in medical technology, the availability of BMT, and the ability to control side effects, more and more children are now undergoing the procedure for many hematologic, oncologic, and metabolic diseases. It will be important to follow each subset of these individuals and see what percentage are fertile and what problems arise related to germline mutation (change in genetic information) after recovering gonadal function following irradiation. Similarly important will be the incidence of abortion or congenital abnormalities in the offspring. The outstanding success of some types of therapy frequently leads to new sets of questions.*

Judith G. Hall, MD

Longitudinal Assessment of Hormonal and Physical Alterations During Normal Puberty in Boys, V: Rising Leptin Levels May Signal the Onset of Puberty

Leptin is a recently described hormone that currently is receiving much attention in relation to both obesity and hypogonadotropic hypogonadism. The authors have studied the changes in leptin concentration with the onset of puberty in 8 normal male children progressing from the prepubertal to adult male state. Comparisons of serum leptin, dehydroepiandrosterone sulfate (DHEAS), testosterone levels, and body mass index (BMI) were carried out every 4 months.

Interestingly, the baseline prepubertal leptin levels were very variable among patients (0.4 ng/mL to 6.0 ng/mL), but in all instances a definite spike in leptin concentration of approximately 2 times base level was noted in the immediate prepubertal period just prior to testosterone rising ≥25 ng/dL, which marks the onset of puberty in the male. By midpuberty the levels had fallen to nearly the prepubertal levels and fell slightly thereafter (see Figure 1). No direct correlation was observed in relation to DHEAS or BMI.

The authors speculate that leptin might be responsible for the nocturnal surges in lateinizing hormone secretion observed with the onset of puberty. They state that there may be a relationship to neuropeptide Y, a target of leptin action in the arcuate nucleus, which positively regulates gonadotropin hormone-releasing hormone secretion in vitro and in vivo.

Mantzoros CS, et al. *J Clin Endocrinol Metab* 1997; 82:1066-1070.

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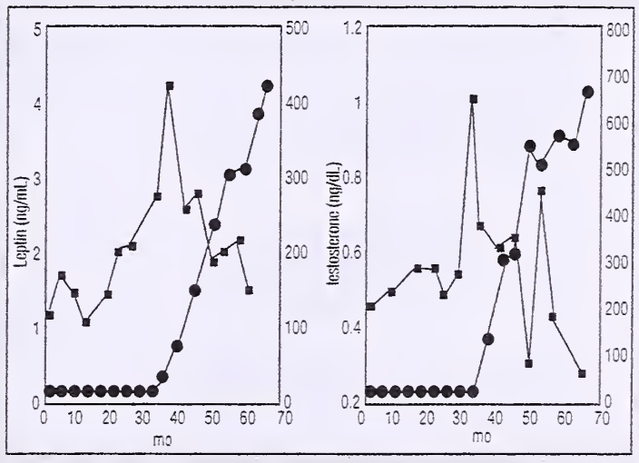
Figure 1



Leptin and testosterone concentration changes over the study period.

From Mantzoros CS, et al. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys, V: rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab* 1997;82(4):1066-1070. ©The Endocrine Society.

Instructions: The Post Self-Assessment/Course Evaluation Answer Sheet can be found on the center page of the issue. Please follow the instructions listed there to receive CME Category 1 credit.

1. Mutations of the type II collagen gene *(COL2A1)* act through a (an) _____ mechanism.
   a. loss of function
   b. gain of function
   c. haploinsufficiency
   d. dominant negative
   e. epigenetic

2. Schmid metaphyseal chondrodysplasia is caused by mutations of:
   a. *COL2A1*
   b. *COL11A2*
   c. *COL10A1*
   d. *FGFR3*
   e. *COMP*

3. Mutations in the diastrophic dysplasia sulfate transporter gene (*DTDST*) are associated with which disorder(s):
   a. achondrogenesis type IB
   b. achondrogenesis type II
   c. atelosteogenesis type II
   d. hypochondrogenesis
   e. a and c

4. Which of the following disorders are caused by mutations that activate transmembrane receptors:
   a. Jansen metaphyseal chondrodysplasia
   b. pseudoachondroplasia
   c. campomelic dysplasia
   d. Kniest dysplasia
   e. a and c

5. Which of the following describes *FGFR3* mutations:
   a. They tend to be dispersed throughout the gene.
   b. They reflect a very high mutability of the gene.
   c. They block signal transduction through this transmembrane receptor.
   d. They account for a small proportion of human chondrodysplasias.
   e. They predispose to mutations of transcription factor genes.

Answer Key: 1. d 2. c 3. e 4. a 5. b

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Drs. Lifshitz, Clarke, Horton, and Hall report no conflicts. Dr. Root serves on Genentech Corporation's National Cooperative Growth Study Advisory Committee. Dr. Blizzard is President of The Genentech Foundation for Growth and Development, which functions independently of Genentech, Inc.

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